The Broader Autism Phenotype in the Parents of Children With Autism Spectrum Conditions

Thesis

The Broader Autism Phenotype in the Parents of Children with Autism Spectrum Conditions

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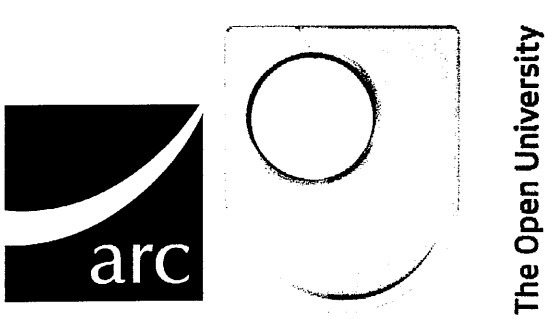

Abstract

Progress in our understanding of the aetiology of Autism Spectrum Conditions (ASC) can be informed by research into the expression of the Broader Autism Phenotype (BAP) in the unaffected genetic relatives of people with ASC. This thesis commences with a comprehensive literature review of the BAP (chapter one), followed by an online study into the BAP in people with ASC, their first-degree relatives and controls focusing on empathy and basic facial emotion recognition (chapter two). Results provide support for the BAP in male first-degree relatives (fathers) who self-reported significantly lower empathy than controls. After setting out the general methods (chapter three), three further empirical studies are described (chapters four to six) that assess whether there are quantitative differences in the expression of autistic traits and related phenotypes in the unaffected parents of simplex (single incidence) and multiplex (multiple incidence) autism families. Multiplex parents were significantly less accurate than simplex parents at attributing mental states to others after controlling for verbal intelligence and performed significantly worse than either simplex parents or controls at identifying specific negative basic emotions from facial expressions. These significant differences in the social domain provide support for the hypothesis that differential genetic mechanisms operate in multiplex and simplex autism. There was also significantly greater aggregation of ADHD traits in multiplex families compared to simplex families, which supports the hypothesis of genetic overlap between ASC and ADHD and bolsters future investigations of cross-syndrome endophenotypes for these conditions. The final empirical study of this thesis (chapter seven) explores the hypothesis that autistic characteristics are 'fractionable' in

ASC parents in ways that are consistent with the DSM-5 defined dyad of behavioural impairments characterizing clinical ASC. The thesis concludes with a summary of findings and implications for future autism research and clinical practice, together with suggestions for future directions in the area of BAP research (chapter eight).

Statement of Work

None of the material in this thesis has been submitted previously for a degree or other qualification to this or any other university or institution. Chapter one is adapted from a published literature review that I wrote on the Broader Autism Phenotype, with contributions from my supervisors (Dr Hoekstra and Dr Roth). Chapter two is also adapted from a published paper, with the co-authors (Dr Allison, Prof Baron-Cohen, Dr Chakrabarti and Dr Hoekstra). This study used online data that is collected and maintained by senior researchers at the Autism Research Centre of the University of Cambridge. I was not involved in designing the measures or collecting/ maintaining the data for the research study described in chapter two. However, I was involved in planning the design of the study in consultation with my primary supervisor (Dr Hoekstra), analysing the data and I was the main author of the paper. The ASC parent and proband data described in chapter three and used for the studies reported in chapters four to seven were collected by me along with four research assistants whom I supervised in the practical aspects of the study. The data collection included three parent-reports about the proband and two performance-based tasks completed by the proband that were not used in this thesis (see chapter three). All other data collected from the ASC parent and proband sample were used in this thesis. The research studies described in chapters three to seven received ethics approval from two research ethics committees: the Human Participants and Materials Ethics Committees (HPMEC) of The Open University and the Cambridge Psychology Research Ethics Committee (CPREC) of the University of Cambridge. I was involved in writing the ethics application to The Open University ethics committee and I wrote the application to the University of Cambridge ethics

committee. The research described in chapters four to six also included a control group that was taken from the same online database of volunteers used in chapter two, which is maintained by senior researchers at the University of Cambridge. The questions behind the research reported in this thesis were developed by me, in consultation with my supervisors (Dr Hoekstra and Dr Roth), my external advisor (Prof Baron-Cohen; chapters three to six) and Dr Allison (chapters three to six). All work presented in this thesis is original and my own. The interpretations are my own, reached through discussions with my supervisors (Dr Hoekstra and Dr Roth).

Publications

Sucksmith, E., Roth, I. & Hoekstra, R.A. (2011). Autistic traits Below the Clinical Threshold: Re-examining the Broader Autism Phenotype in the 21st Century. *Neuropsychology Review*, 21(4), 360-389.

Sucksmith, E., Allison, C., Baron-Cohen, S., Chakrabarti, B. & Hoekstra, R.A. (2013). Empathy and emotion recognition in people with autism, first-degree relatives and controls. *Neuropsychologia*, 51 (1), 98-105.

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Sucksmith, E., Roth, I., Allison, C., Baron-Cohen, S. & Hoekstra, R.A. (in preparation). Exploring psychiatric history and parental psychopathology in multiplex versus simplex autism families.

Acknowledgements

The roots of my interest in autism research can be traced back to my experiences as a care worker for a charity in Cheltenham in 2007/08. My manager had given me the ostensibly simple task of helping an elderly man with high-functioning autism called Michael to move house. The stress and anxiety of living in a new, unpredictable environment surrounded by new people transpired to be too great for Michael and he was unable to successfully make the transition. My experiences supporting Michael sparked my interest in autism by exposing me to the profound problems that some people face interacting and communicating with others in the real world, sometimes in spite of possessing exceptional abilities - Michael could translate English books into several different languages, yet struggled to grasp simple social remarks and greetings or could not work out when it was his turn to speak on the telephone. I only hope that this thesis will contribute a small, but significant, piece of the jigsaw to understanding this complex neurodevelopmental condition so that we can improve our understanding and support for people like Michael.

I would like to express my thanks to The Open University for providing me with a 3 year PhD studentship, which gave me the financial support I needed to conduct the research described in this thesis. In addition, I owe a huge debt of gratitude to a number of people, without whom this thesis would not have been possible. First and foremost, I would like to thank my primary supervisor, Dr Rosa Hoekstra, whose support, guidance and feedback throughout my PhD has been immense. I count myself as very lucky to have

such an inspirational, dedicated and talented supervisor to keep me on the right track. Secondly, I would like thank my second supervisor, Dr Ilona Roth for all her advice and encouragement over the last three years and for sharing her wealth of experience and knowledge of autism research with me. Thank you Rosa and Ilona for giving me the opportunity to conduct this PhD project and for believing in me!

Thirdly, I would like to wholeheartedly thank colleagues at the Autism Research Centre of Cambridge University. I spent three wonderful years working at the ARC and I am incredibly grateful for the chance to work alongside such talented and knowledgeable autism researchers. In particular, I would like to thank my external advisor, Professor Simon Baron-Cohen, for giving me the opportunity to work at the Cambridge ARC and to let me participate in the research centre's social and academic activities. I also record my deep sense of gratitude for his superb theoretical guidance along the way. I would also like to thank Dr Carrie Allison for all her help in the design of the empirical studies, recruiting participants and managing the ARC volunteer database, which was used for the study in chapter two. In addition to Prof. Baron-Cohen and Dr. Allison, I would also like to express my thanks to the following collaborators and colleagues for their help with this thesis: Dr Bhismadev Chakrabarti for his help with chapter two and for designing the KDEF task, Jon Breidbord for his advice on experimental analysis of the KDEF using the ARC online interface, Dr Will Mandy and Hannah Gordon at University College London for their help in double-rating the 3Di-short parental interview, and Dr Payam Rezaie and Dr Antonio Martins-Mourao for their comments on an earlier draft of chapter one. I would also like to thank the four research assistants who helped recruit participants,

accompanied me on home visits, and collected/ entered data: Diana Deca, Francesca Cabedo, Maaike Hoeksma and Leanne Swain. I am immensely grateful for their dedication and hard work during the home visits; for their excellent company on long, arduous journeys (in an old, temperamental car!) and their cheerful demeanour irrespective of the circumstances (including the grey, drizzly English weather!).

My friends in Cambridge were a great source of support for me during my PhD thesis; in particular, I would like to thank Amber Ruigrok, Renate Van De Ven, Teresa Tavassoli and all my other friends at the ARC. Special thanks also go to my parents, Linda and Peter, and my girlfriend, Lisa, for their unconditional support and encouragement throughout. I couldn't have done it without you!

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Abbreviations

ADHD: Attention Deficit/ Hyperactivity Disorder

ADI-R: Autism Diagnostic Interview-Revised

ADOS: Autism Diagnostic Observational Schedule

ADOS-G: Autism Diagnostic Observational Schedule-Generic

ANOVA: Analysis of Variance

ANCOVA: Analysis of Covariance

AQ: Autism-Spectrum Quotient

ART: Accuracy-adjusted Response Time

ASC: Autism Spectrum Conditions

ASR: Adult Self-Report form

BAP: Broader Autism Phenotype

BAPQ: Broad Autism Phenotype Questionnaire

BDT: Block Design Task

BPASS: Broader Phenotype Autism Symptom Scales

BPVS: British Picture Vocabulary Scale

CNV: Copy Number Variations

DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision

DTI: Diffusion Tensor Imaging

EFT: Embedded Figures Task

EQ: Empathy Quotient

ERP: Event-Related Potential

FFSF: Face to Face/ Still Face task

FHI: Autism Family History Interview

fMRI: functional Magnetic Resonance Imaging

HFA: High-Functioning Autism

KDEF: Karolinska Directed Emotional Faces task

ICD-10: International Classification of Diseases, 10th Revision

ID: Intellectual Disability

IQ: Intelligence Quotient

MANCOVA: Multivariate Analysis of Covariance

MEG: Magnetoencephalography

PDD: Pervasive Development Disorder

PDD-nos: Pervasive Development Disorder- not otherwise specified

PIQ: Performance Intelligence Quotient

PRS: Pragmatic Rating Scale

RAN: Rapid Automatised Naming task

RPM: Raven's Progressive Matrices

RT: Response Time

SADS-L: Schedule for Affective Disorders and Schizophrenia-Lifetime Version

sMRI: structural Magnetic Resonance Imaging

SNV: Single Nucleotide Variants

SQ-R: Systemising Quotient-Revised version

SRS: Social Responsiveness Scale

ToM: Theory of Mind

VIQ: Verbal Intelligence Quotient

3Di-short: The Developmental Dimensional and Diagnostic Interview-Short version

Introduction

Autism Spectrum Conditions (ASC) and their aetiology

Autism Spectrum Conditions (ASC)[1] refer to a set of neurodevelopmental conditions that lead to abnormalities in social interaction, communication and an atypically restrictive and repetitive repertoire of interests and activities (APA, 2000; WHO, 1993). Collectively these symptom domains are called the 'triad of impairments'. Throughout this thesis the terms 'autism' and 'ASC' are equivalent and describe the following sub-categories: autistic disorder, Asperger syndrome and pervasive development disorder not otherwise specified (PDD-nos; APA, 2000; see Table I).

Table I: *Sub-categories of Autism Spectrum Conditions and their distinguishing features according to the Diagnostic and Statistical Manual of Mental Disorders- 4th edition-text revision (DSM-IV-TR; APA, 2000)*[2]

Sub-categories	Distinguishing features
Autistic disorder	Impairments in all three areas of the 'triad' and abnormal development before 3 years of age
Asperger syndrome	Impairments in all three areas of the 'triad', no language delay before 3 years of age, no significant delay in cognitive development
PDD-nos	Meeting autism criteria but showing a late age of onset (> 3 years) or individuals who show severe impairment in only one or two core areas of the 'triad', with or without cognitive or language delay

[1] The term 'autism spectrum condition' is preferred to the term 'autism spectrum disorder' in this thesis because the behaviours that this term is describing may not necessarily result in disability and there are a number of positive as well as negative aspects to autism that can be embraced and celebrated. By using the term 'condition', which is considered to be a more neutral term than 'disorder', this acknowledges that autism is considered as a set of cognitive differences that need social acceptance and support, rather than solely a harmful disability that needs to be cured.

[2] ICD-10 offers a similar, though not identical sub-classification.

Early hypotheses about the aetiology of ASC largely posited environmental influences, such as deficient socio-emotional child-rearing strategies (Bettelheim, 1967) or an emotionally and sustained failure in parent-child interactions (Tinbergen, 1973). However, landmark twin and family pedigree studies starting in 1977 by Folstein and Rutter have confirmed that ASC have a significant genetic component with a heritability estimate of 90% (Piven et al., 1997; Rutter, 2000; Skuse, 2007). Studies analysing the differences in concordance rates between monozygotic (MZ) and dizygotic (DZ) twins have been critical for determining the relative contribution of genetic and environmental factors to the aetiology of ASC. These studies have shown that the concordance rates for autistic disorder in MZ twins is much higher than DZ twins (e.g. 60% MZ versus 5% DZ pairs reported by Bailey et al., 1995), suggesting that autism has a genetic aetiology (Folstein and Rutter, 1977; Bailey et al., 1995). These early twin studies used a narrow definition of autism. More recent studies have confirmed that the same is true when autism is reconceptualised as a spectrum of conditions; for example, Taniai et al. (2008) and Rosenberg et al. (2009) reported high concordance rates for ASC in MZ twins (88-95%) but only modest concordance rates in DZ twins (both 31%). Furthermore, when Folstein and Rutter adopted a broader definition of the autism phenotype in their 1977 landmark study, concordance rates in MZ twins rose from 36% to 82%, which is very similar to present rates. Similarly, family studies support a genetic aetiology of ASC, reporting recurrence risk in the relatives of ASC probands that is several fold greater than the risk of ASC in the general population (e.g. Losh et al., 2008, Ozonoff et al., 2011)[3].

[3] The exact increased risk of autism in relatives compared to the general population risk varies in the literature and depends on the latest prevalence estimates and the definitional criteria used for diagnosing autism

Autism symptomatology can be split into two categories according to whether the aetiology is known (non-idiopathic) or unknown (idiopathic). Autistic traits are observed in a number of known genetic and chromosomal disorders, including Fragile X Syndrome, Tuberous Sclerosis, Rett Syndrome, Turner Syndrome, William's Syndrome and Down Syndrome (Skuse, 2007). Cytogenetic lesions are found in approximately 6-7% of ASC cases, such as inherited duplication of the chromosomal region 15q11-15q13 (Abrahams and Geschwind, 2008). These non-idiopathic cases constitute approximately 10% of all individuals with autism, whilst the remaining 90% have idiopathic autism (Geschwind, 2008). If unclassified, rare de novo mutations are taken into account, then the percentage of non-idiopathic cases may be as large as 20% (Abrahams and Geschwind, 2008). Researchers disagree as to whether these cases of non-idiopathic autism should be included *within* the autism spectrum. However, research into the Broader Autism Phenotype (see next) usually focuses on families affected by *idiopathic* autism.

Thesis outline

Despite considerable progress in understanding ASC over the past few decades, more research is needed into discerning the biomedical aetiology of ASC and the related factors that make the autism spectrum so heterogeneous. One way of providing insights into these outstanding issues in autism research is to study the autism phenotype in the genetic, first-degree relatives of people diagnosed with ASC. Relatives of people with ASC often show milder expression of traits that are characteristic of ASC, also referred to

as the 'Broader Autism Phenotype' (BAP; Constantino et al., 2006; Rutter, 2000). It is believed that the BAP may reflect the wider genetic liability to ASC and could be useful in identifying phenotypes that are under stronger genetic influence than the clinical phenotype.

Before conducting empirical research into the BAP, it is crucial to look back on family studies of ASC and assess what is currently known about the BAP and to identify the most promising avenues for further research. This thesis commences with a comprehensive literature review of the BAP (chapter one), which aims to identify the candidate phenotypic traits delineating its boundaries. This review concludes by putting forward the experimental rationale for the studies reported in this thesis; this includes the reason for exploring the BAP by focusing on self-report scales and performance-based measures of empathy and the reason for studying differences in the expression of the BAP in ASC parents stratified according to their affiliation to multiplex and simplex families. Chapter one is followed by an online empirical study into the BAP in adults with ASC, their first-degree relatives and controls using a self-rated scale of empathy and a measure of basic facial emotion recognition (chapter two). This thesis then goes on to examine whether the BAP is mainly restricted to specific groups of ASC relatives. After setting out the general methods (chapter three), three further empirical studies are described (chapters four to six) that assess whether there are quantitative differences in the expression of autistic traits and related phenotypes in the 'unaffected' parents of simplex and multiplex families, using a battery of behavioural and cognitive measures. Studies into the expression of autistic traits and related phenotypes in ASC parents

conclude in chapter seven, which explores the hypothesis that social and non-social autistic characteristics are 'fractionable' in ASC parents in ways that are consistent with the DSM-5 defined dyad of behavioural impairments characterizing clinical ASC. The thesis concludes with a summary of findings and implications for future autism research and clinical practice, together with suggestions for future directions in the area of BAP research (chapter eight).

Chapter One

Autistic traits below the clinical threshold: a review of the literature on the Broader Autism Phenotype[4]

[4] This chapter is adapted from: Sucksmith, E., Roth, I. & Hoekstra, R.A. (2011). Autistic traits Below the Clinical Threshold: Re-examining the Broader Autism Phenotype in the 21st Century. *Neuropsychology Review*, 21(4), 360-389.

1.1 Abstract

Diagnosis, intervention and support for people with ASC can be assisted by research into their aetiology. Twin and family studies indicate that ASC are highly heritable; genetic relatives of people with ASC often show milder expression of traits characteristic for ASC, referred to as the BAP. In the past decade, advances in the biological and behavioural sciences have facilitated a more thorough examination of the BAP from multiple levels of analysis. In this chapter the candidate phenotypic traits delineating the BAP are summarised, including key findings from neuroimaging studies examining the neural substrates of the BAP. After summarising the literature, this chapter emphasises the importance of exploring differences in the expression of the BAP in multiplex versus simplex autism families. This chapter also stresses the need to derive heritable endophenotypes that will reliably index ASC susceptibility and offer neurodevelopmental mechanisms to bridge the gap between genes and a clinical ASC diagnosis. The chapter concludes by highlighting some important remaining research into the BAP, which are empirically explored in chapters two to seven.

1.2 Introduction

1.2.1 Historical Background

The 'Broader Autism Phenotype' (BAP) is a term describing a group of 'sub-threshold' social skills and communication traits and unusual personality features that are frequently found in the relatives of people with ASC and which are believed to be milder manifestations of traits characteristic for clinically diagnosed ASC (Constantino et al., 2006; Rutter, 2000). The BAP concept derives from observations made in the 1940s by Leo Kanner and Hans Asperger, who reported behavioural features in parents that were similar in kind to those of their autistic offspring. For example, in Kanner's case studies of children with 'autistic psychopathy' in 1943, both first and second-degree relatives were selectively described as late speakers, mildly obsessive and uninterested in people (Kanner, 1943). Likewise, Asperger described a subset of parents of autistic children as withdrawn, pedantic, eccentric and loners who had problems relating to the outside world (Asperger, translated by Frith, 1991). Thus from a very early period, observations suggested that the expression of autistic traits extends beyond the clinical boundaries of ASC to include a mild sub-threshold expression in relatives, supporting the hypothesis that the aetiology of ASC include a significant genetic component.

It has been over 12 years since the BAP was first comprehensively reviewed (Bailey et al., 1998). In over a decade since this review was written, there have been substantial advances in the methodological tools used by researchers to study the BAP. In the last 10

years, various researchers (e.g. Baron-Cohen et al., 2001b; Constantino et al., 2006; Hoekstra et al., 2008) advanced the notion that, rather than a discrete category, the phenotype of ASC can be conceptualised as a set of continuous, quantitative traits that merge into the general population. This has been accompanied by the development of new psychometric scales, such as the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001b) and the Social Responsiveness Scale (SRS; Constantino, 2002) which have allowed sub-threshold autistic traits to be measured more precisely. The last decade has also seen a wider availability of brain scanning techniques, which have allowed the structure and function of the brain to be examined more directly in individuals diagnosed with ASC, their relatives and control groups. The results and conclusions of brain scanning experiments are also beginning to dramatically improve our understanding of the neural underpinnings of the BAP. This chapter therefore provides an up-to-date summary of research findings on the BAP in the fields of psychology, cognitive neuroscience and related disciplines.

1.2.2 Measuring and defining the BAP: methodological considerations

In 1977, Folstein and Rutter's pioneering study of concordance for autism in monozygotic (MZ) and dizygotic (DZ) twins provided a pattern of findings consistent with a broader phenotype for autism (Folstein and Rutter, 1977). Since then, researchers have explored the BAP using a variety of measures and research designs. Before setting out the research findings of the different studies, it is important to highlight some key differences in the methods used. Firstly, several early family studies supporting the

presence of a broader phenotype in the parents and/ or siblings of autistic probands were heavily reliant on qualitative, categorical data collected from observational reports and interviews (e.g. Bolton et al., 1994; Gillberg, 1989; Landa et al., 1992; Piven et al., 1994; Piven and Palmer, 1999; Wolff et al., 1988). These studies used a discrete measure of the BAP; similar to a discrete ASC diagnosis, the BAP was either present or absent. With the development of scales such as the AQ and the SRS, the characteristics of the BAP can now be assessed quantitatively.

As well as a shift from dichotomous to quantitative measures, methodology has differed in terms of which participants are included in studies on the BAP. Most studies focus on relatives of people with ASC who do not have a clinical ASC diagnosis themselves. As such, they are clinically 'unaffected' with ASC. However, not all studies have excluded affected relatives (e.g. Virkud et al., 2009), making it difficult to evaluate whether average elevated autistic traits can simply be ascribed to this clinical subgroup of the sample (see Hoekstra and Wheelwright, 2010 for discussion). Some studies analyse the BAP in the infant siblings of children diagnosed with ASC. For example, Holmboe et al. (2010) explored attentional disengagement and selective inhibition problems in infant siblings of autistic probands. Other studies focusing on 'at-risk' infant siblings include Cassel et al. (2007), Merin et al. (2007), Presmanes et al. (2007) and Toth et al. (2007) (see Table 1.1, which summarises a range of research studies examining autistic traits in the infant siblings of autistic probands). Whilst components of the phenotype of ASC can be found in this experimental group, it is not clear whether these are features of the BAP or early indicators of the full phenotype of ASC, since a reliable diagnosis can not be

given yet. Whether these children are truly 'unaffected' with clinical ASC and display early sub-threshold expression of autistic traits or are children who may later receive an autism spectrum diagnosis is thus uncertain using this methodological design. Other researchers in turn have used more liberal participant selection criteria, choosing to examine autistic traits in the general population rather than in relatives of people with ASC (e.g. Jobe and White, 2007).[5] Still other researchers have extremely conservative selection criteria, splitting up the genetic relatives of autistic probands into 'BAP+' and 'BAP-' groups following one or more discrete criteria, and measuring autistic traits in the 'BAP+' group only (e.g. Adolphs et al., 2008; Losh et al., 2009) rather than analysing average differences amongst all genetic relatives taken together (e.g. Dalton et al., 2007).

In addition, studies compare the relatives of autistic probands with different types of control groups. Some researchers have used a clinical control group, such as parents of children with Down Syndrome (e.g. Piven et al., 1997b; Ruser et al., 2007) or Specific Language Impairment (e.g. Lindgren et al., 2009) which helps to eliminate confounding variables associated with caring for a child with special needs. In contrast, some research studies use a non-clinical control group; the genetic relatives of typically developing individuals who do not have any psychiatric conditions (e.g. Losh et al., 2009). In some studies these comparison groups have been well-matched on variables such as age, sex and IQ (e.g. Dorris et al., 2004; Wong et al., 2006) but less so in others (e.g. Piven and Palmer, 1997).

[5] in this chapter the discussion of the BAP is restricted to studies conducted in the relatives of people with autism.

Finally, there are a variety of advantages and disadvantages to using different types of measures to detect the BAP. Interviews are an extremely effective means of examining autistic traits and related phenotypes across a participant's life span, but are often time-consuming and stressful for participants whilst the capacity for researchers to accurately rate answers is constrained by the quality of the participant's verbal response. Observational assessments allow researchers to assess behaviour first-hand free from biased responses from informants, but are constrained by a small period of time in which to observe autistic characteristics in the participant and observations are restricted to specific circumstances and contexts. Performance-based cognitive tasks are also more objective measures of ASC-related phenotypes, but may be confounded by variables such as IQ and motivation and are only a 'snapshot' of a participant's functioning at a particular point in his/her development. Finally, self or informant-rated questionnaires are used in BAP studies, which are quick and less stressful for participants than other measures, and can be completed by participants in their own time. However, informant and especially self-rated questionnaires are subjective measures where participants may give inaccurate or socially desirable answers to questionnaire items. Whichever measure is used to detect the BAP it is recommended that it has a number of the following properties: (1) be a quantitative measure with a wide range of scores so it is sensitive to detecting subtle differences that are indicative of the BAP, (2) have good content validity by distinguishing participants with and without ASC, (3) be applicable across participants' entire developmental period from childhood to adulthood, (4) have good test-retest reliability and (5) have good concurrent validity by correlating with other biological or psychological measures of the BAP.

It is important to bear these methodological differences in mind when reading the findings presented in this review. Since Folstein and Rutter's landmark twin study in the 1970s, there have been a number of family and twin studies looking for autism-related characteristics in the relatives of probands, which have achieved mixed success. Here, these candidate traits are examined at different levels of analysis, starting with the behavioural (including the 'three domains of impairment' (DSM-IV-TR; APA, 2000) defining the narrower phenotype of autism). This level is assessed using interviews, observational assessments and self/ other-report questionnaires, which explore the expression of autistic traits in naturalistic contexts. The chapter then examines the BAP from the cognitive level (e.g. atypical social cognition, executive function and visual attention) using performance-based measures that systematically examine brain functioning in experimentally controlled settings. Finally this chapter summarises neuroimaging studies investigating possible neuroanatomical and neurofunctional correlates of the BAP. The overview that follows comes with the caveat that there is strong overlap between the 'behavioural' and 'cognitive' levels to the extent that some behavioural measures described could also be considered cognitive and vice-versa. Furthermore, within the 'behavioural' level of impairments there is strong overlap between the domains of 'reciprocal social interaction' and 'language and communication'. Therefore some traits that are here included in the domain of 'reciprocal social interaction' may also be included in the domain of 'language and communication' and vice-versa.

The candidate traits that are examined also depend on the stage of development that the participants are sampled. For example, a number of studies have examined early social behaviours such as joint attention, requesting, eye gaze movements and play behaviour in the younger infant siblings of children with ASC (e.g. Landa et al., 2007; Merin et al., 2007; Toth et al., 2007). Other studies have focused on later social behaviour in older relatives of people with ASC, such as empathic understanding, social expressiveness and social motivation (e.g. Szatmari et al. 2008; Dawson et al., 2007). Isolated traits appearing early in human development may serve as important precursors for the emergence of traits at a later stage in development. Therefore a distinction is made here between an 'early' BAP arising in the 'at-risk' infant siblings of children with ASC and a 'later' BAP present in the older relatives of people with ASC. To aid the reader in the following sections, a summary of the traits discussed in the early and later BAP has been provided in Tables 1.1 and 1.2 respectively (for a summary of demographic information for these studies, see Appendix 1.1 and 1.2).

Table 1.1: *An early emerging BAP? A summary of research studies reporting autistic traits in the 'at-risk' infant siblings of autistic probands.*

	Category	Candidate Traits	Support in research literature[6] (number of studies reviewed)	Measures used (examples)
Behavioural level	1. Language and communication	• Semantic-pragmatic language	++ (1)	• Autism Diagnostic Observational Schedule (Ben-Yizhak et al., 2011)
		• Language delay	sig. levels not reported (3)	
		• Rate of communicating	++ (1)	• Communication and Symbolic Behavior Scale Developmental Profile (Toth et al., 2007)
	2. Motor development	• Reduced time spent in different postures	++ (1)	• Videotape rating of posture bouts (Iverson and Wozniak, 2007)
	3. Social interaction	• Response to name	-/+ (2)	• Responding to Name task (Nadig et al., 2007)
		• Initiation of Joint Attention	+ (3)	• Early Social Communication Scales (Goldberg et al., 2005)
		• Response to Joint Attention	+ (5)	• Responding to Joint Attention task (Presmanes et al., 2007)
		• Joint attention (combined)	-- (1)	• Communication and Symbolic Behavior Scale Developmental Profile (Toth et al., 2007)
		• Reduced requesting behaviours	+ (4)	• Early Social Communication Scales (Goldberg et al., 2005)
		• Response to Social Interaction	++ (1)	• Early Social Communication Scales (Goldberg et al., 2005)
		• Atypical gaze shifting	-/+ (5)	• The face-to-face/ still-face paradigm (Cassel et al., 2007; Merin et al., 2007)
		• Reduced social smiling/ higher rates of 'neutral affect' during FFSF task	+ (3)	• The face-to-face/ still-face paradigm (Cassel et al., 2007; Merin et al., 2007)
		• Weaker mother-infant synchrony for infant-led interactions during free play	++ (1)	• Coding of mother-infant free play interactions (Yirmiya et al., 2006)

[6] scoring system approximately indicates the percentage of studies reviewed that report statistically significant differences between autism relatives and clinical/ non-clinical control groups; ++ = 80-100 %; + = 60-80 %; -/+ = 40-60 %; - = 20-40 %; -- = 0-20%

	Category	Candidate Traits	Support in research literature (number of studies reviewed)	Measures used (examples)
		• Reduced gaze towards caregiver's eyes, relative to mouth	++ (1)	• The face-to-face/ still-face paradigm (Cassel et al., 2007; Merin et al., 2007)
		• Reduced symbolic behaviour during free play	-/+ (2)	• Communication and Symbolic Behavior Scale Developmental Profile (Toth et al., 2007)
		• Fewer distal gestures (e.g. pointing)	++ (1)	• Communication and Symbolic Behavior Scale Developmental Profile (Toth et al., 2007)
		• Imitation difficulties	-- (1)	• Battery of imitation tasks (Toth et al., 2007)
		• Reduced functional play behaviour	-- (1)	• Coding of free-play assessment (Christensen et al., 2010)
	4. Repetitive, restrictive behaviours and interests	• Higher non-functional repeated behaviours	++ (1)	• Coding of free-play assessment (Christensen et al., 2010)
Cognitive level	5. Social cognition	• 'Theory of Mind' understanding	-- (1)	• Strange Stories Task (Shaked et al., 2006) • False Belief Task (Shaked et al., 2006)
	6. Executive function	• Enhanced working memory for non-social targets	++ (1)	• Modified 'Peekaboo' Game (Noland et al., 2009)
	7. Visual attention	• Attentional disengagement	-/+ (2)	• Visual orienting task (Elsabbagh et al., 2009b)
		• Difficulties automatically orienting to targets/ forming expectations about visual environment	++ (1)	• Visual orienting task (Elsabbagh et al., 2009b)
		• Face processing: increased attention to mouth, relative to eyes	++ (1)	• The face-to-face/ still-face paradigm (Ibanez et al., 2008; Merin et al., 2007)
	8. Language ability	• Receptive/ expressive language abilities	+ (6)	• The Mullen Scales of Early Learning (Toth et al., 2007) • Clinical Evaluation of Language Fundamentals for pre-school children (Levy and Bar Yuda, 2011)
	9. Contrast sensitivity	• Luminance contrast sensitivity	++ (1)	• Forced-Choice Preferential Looking Paradigm (McCleery et al., 2007)
	10. General Cognitive abilities	• Delays in general cognitive development	-- (5)	• Bayley Scales of Infant Development, 2nd Edition (Yirmiya et al., 2007)

Table 1.2: *Candidate traits constituting the BAP in older relatives.*

	Category	Candidate Traits	Support in research literature[7] (number of studies reviewed)	Measures used (examples)
Behavioural level	1. Language and communication	• Pragmatic difficulties	++ (8)	• Pragmatic Rating Scale (Losh et al., 2008)
		• Broadly defined communication difficulties	++ (9)	• Autism Family History Interview (Folstein et al., 1999; Pickles et al., 2000; Piven et al., 1997a)
		• Structural language problems	-/+ (2)	• Children's Communication Checklist-2 (Bishop et al., 2006)
		• Reading/ writing/ spelling and articulation problems	-/+ (5)	• Autism Family History Interview (Folstein et al., 1999; Pickles et al., 2000; Piven et al., 1997a)
		• Difficulties engaging in spontaneous narrative discourse	++ (1)	• Narrative discourse task (Landa et al., 1991)
	2. Social interaction	• Broadly defined social difficulties	++ (4)	• Autism Family History Interview (Folstein et al., 1999; Pickles et al., 2000; Piven et al., 1997a)
		• Alexithymia	++ (1)	• Toronto Alexithymia Scale-20 (Szatmari et al., 2008)
		• Reduced quality/ number of social relationships	++ (4)	• The Friendship Interview (Losh and Piven, 2007)
		• Reduced social motivation	no control groups (1)	• Broader Phenotype Autism Symptom Scale (Dawson et al., 2007)
		• Reduced social expressiveness	no control groups (1)	• Broader Phenotype Autism Symptom Scale (Dawson et al., 2007)
		• Reduced social responsiveness	++ (2)	• The Social Responsiveness Scale (Constantino et al., 2006)
		• Poor social skills	++ (6)	• Autism-Spectrum Quotient (Bishop et al., 2004)
		• Reduced social engagement	++ (1)	• Communication Checklist – Adult Version (Whitehouse et al., 2010)

[7] scoring system approximately indicates the percentage of studies reviewed that report statistically significant differences between autism relatives and clinical/ non-clinical control groups; ++ = 80-100 %; + = 60-80 %; -/+ = 40-60 %; - = 20-40 %; -- = 0-20%

	Category	Candidate Traits	Support in research literature (number of studies reviewed)	Measures used (examples)
	3. Repetitive, restrictive behaviours and interests	• Rigidity	+ (5)	• Modified Personality Assessment Schedule-Revised (Losh et al., 2008)
		• Circumscribed interests	- - (2)	• Clinical interview (Wolff et al., 1988)
		• Broadly defined stereotyped behaviours	+ (4)	• Autism Family History Interview (Piven et al., 1997a)
		• Reports of real-life non-social skills and preferences	+ + (1)	• Real life styles and preferences questionnaire (Briskman et al., 2001)
Cognitive level	4. Social cognition	• Theory of Mind ability	+ (7)	• Mind in Eyes Task (Baron-Cohen and Hammer, 1997)
		• Emotion recognition	+ (7)	• Emotion Recognition Test (Bölte and Poustka, 2003)
		• Trustworthiness of faces	+ + (1)	• Trustworthiness of Faces Task (Losh et al., 2009)
		• Discerning emotional content of complex social scenes	+ + (1)	• Movie Stills Task (Losh et al., 2009)
		• Differences in face processing strategy	+ + (1)	• 'Pictures of Facial affect'/ 'Bubbles' Task (Adolphs et al., 2008)
		• Face recognition/ memory ability	- / + (4)	• Facial Recognition Task (Dalton et al., 2007)
		• Eye gaze processing/ social orienting difficulties	+ + (2)	• Directional Judgement Task (Wallace et al., 2010)
	5. Executive Function	• Mental flexibility/ set-shifting	- (9)	• Wisconsin Card Sorting Test (Bölte and Poustka, 2006)
		• Reduced planning ability	- / + (9)	• Tower of Hanoi (Losh et al., 2009)
		• Ideational Fluency	+ + (1)	• Pattern meanings (Wong et al., 2006)
		• Verbal fluency	- (5)	• FAS Verbal Fluency Task (Hughes et al., 1999)
		• Design fluency	- / + (2)	• The Design Fluency Task (Delorme et al., 2007)
		• 'Association fluency'	- - (1)	• The Association Fluency Task (Delorme et al., 2007)
		• Inhibition/ working memory problems (verbal/ spatial)	- (6)	• Delayed Oculomotor Response Task (Koczat et al., 2002)
		• Spatial span	+ (3)	• Spatial span task (Hughes et al., 1999)

Category	Candidate Traits	Support in research literature (number of studies reviewed)	Measures used (examples)
6. Visual attention, sensory integration and sensorimotor functioning	• local attentional biases/ 'weak central coherence'	- / + (6)	• Embedded Figures Task (Happé et al., 2001)
	• Susceptibility to visual illusion	+ + (1)	• Titchener Circles Illusion (Happé et al., 2001)
	• 'Complex' divided, selective attention/ selective inhibition	+ + (1)	• Visual, divided attention task (Belmonte et al., 2010)
	• Attentional engagement/ disengagement	+ + (1) + + (1)	• The Detection Task (Scheeren and Stauder, 2008)
	• Oculomotor abnormalities (e.g. open-loop pursuit gain)		• Saccade and foveofugal step-ramp tasks (Mosconi et al., 2010)
7. Language ability	• Phonological Processing	- (3)	• The Comprehensive Test of Phonological Processing (Lindgren et al., 2009)
	• Rapid Automatised Naming (RAN)	- / + (4)	• Object and Colour naming tasks (Losh et al., 2010)
	• Receptive and expressive language ability	- - (3)	• The Clinical Evaluation of Language Fundamentals, 3rd Edition (Lindgren et al., 2009, Pilowsky et al., 2003)
	• Reading ability	+ (5)	• The Woodcock-Johnson Psycho-Educational Battery-Revised (Lindgren et al., 2009)
	• Spelling ability	- / + (5)	• The Schonell Graded Word Spelling Test-B (Fombonne et al., 1997)
8. Motion Perception	• Luminance contrast sensitivity/ 'atypical Magnocellular pathway functioning'	- / + (2)	• Detection and Motion Tasks (Koh et al., 2010)
9. General Cognitive abilities	• Intellectual functioning/ disability	- / + (12)	• WAIS-R/ WISC-R (Fombonne et al., 1997)

	Category	Candidate Traits	Support in research literature (number of studies reviewed)	Measures used (examples)
Psychiatric history	10. Other Psychiatric Conditions	• Depression/ affective disorder	++ (10)	• The Maudsley SADS-L (Bolton et al., 1998)
		• Social phobia	++ (2)	• Schedule for Affective Disorders and Schizophrenia (Piven and Palmer, 1999)
		• Anxiety disorder	+ (4)	• Self-report and GP medical records on family psychiatric history (Micali et al., 2004)
		• OCD	++ (6)	• Autism Family History Interview (Bolton et al., 1998)
		• Motor tics	-/+ (2)	• Autism Family History Interview (Bolton et al., 1998)
		• Schizophrenia	-- (2)	• Autism Family History Interview (Ghaziuddin, 2005)
		• Alcoholism	-- (2)	• Autism Family History Interview (Piven & Palmer, 1999)
Personality Traits	11. Personality Traits	• Rigid	+ (5)	• The Modified Personality Assessment Schedule-Revised (Losh et al., 2008, 2009, Murphy et al.. 2000)
		• Impulsive	-/+ (2)	• The Modified Personality Assessment Schedule-Revised (Losh et al., 2008, 2009; Murphy et al., 2000)
		• Aloof	++ (5)	• The Modified Personality Assessment Schedule-Revised (Losh et al., 2008, 2009; Murphy et al., 2000)
		• Shy	-/+ (2)	• The Modified Personality Assessment Schedule-Revised (Losh et al., 2008, 2009; Murphy et al., 2000)
		• Untactful	-/+ (4)	• The Modified Personality Assessment Schedule-Revised (Losh et al., 2008, 2009; Murphy et al., 2000)
		• Schizoid/ reserved	++ (2)	• Personality Style and Disorder Inventory (Bölte et al., 2007)
		• Irritable	-/+ (2)	• The Modified Personality Assessment Schedule-Revised (Piven et al., 1994)
		• Hypersensitive	+ (5)	• The Modified Personality Assessment Schedule-Revised (Piven et al., 1994)
		• Neuroticism	++ (2)	• The NEO personality inventory (Losh et al., 2008)
		• Undemonstrative	- (3)	• The Modified Personality Assessment Schedule-Revised (Piven et al. 1994)
		• Anxious	+ (4)	• The Modified Personality Assessment Schedule-Revised

1.3 A Review of BAP research studies

1.3.1 Behavioural level

1.3.1.1 Language and communication

Characteristics pertaining to the language domain of autistic atypicalities have been extensively studied in the relatives of people with ASC. Research findings suggest that parents and siblings of autistic probands have significantly greater difficulty using language to communicate for social purposes (pragmatics) compared to controls (see Tables 1.1 and 1.2). For example, the infant siblings of children with ASC identified with the BAP using the scores of items taken from the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2002) scored poorly on semantic-pragmatic language compared to typically developing infants (Ben-Yizhak et al., 2011). Pragmatic difficulties have also been found in adult relatives e.g. the parents of children with ASC scored poorly on the 'pragmatic skills' subscale of a self-report questionnaire called the 'Communication Checklist-Adult Version' (Whitehouse and Bishop, 2009) compared to controls from the general population. However, this group difference did not reach statistical significance (Whitehouse et al., 2010). Similarly, the parents of autistic probands categorised as 'aloof' tended to have greater problems with pragmatic language use, as indicated by an interview-based performance measure called the Pragmatic Rating Scale (PRS; Landa et al., 1992; Losh and Piven, 2007). Studies by Bishop et al. (2004) and Whitehouse et al. (2007) assessed the language abilities of parents of children with

ASC and found significantly higher average levels of pragmatic difficulties compared to both clinical and non-clinical control groups, as indicated by the communication and social subscales of the AQ. An additional study conducted by the same research group found associations between the same two combined subscales of the AQ in fathers and children scoring low on the Children's Communication Checklist-2 (Bishop, 2003; Bishop et al., 2006). Similar findings have also been reported using large sample sizes by Wheelwright et al. (2010) and in a cross-cultural validation study of the BAP using clinical and non-clinical samples from Italy (Ruta et al., 2011). In both studies, the parents of children with ASC scored significantly higher than a control group for difficulties on the communication subscale of the AQ.

Other family studies examining the communication domain used a modified version of the PRS; both Piven et al. (1997b) and Ruser et al. (2007) found that the parents of probands with ASC had significantly lower scores on this measure than a clinical control group (parents of Down Syndrome children). This was especially true for the male relatives of autistic probands who displayed poor social-pragmatic abilities, as measured by the modified PRS. However, the lower communication abilities found were not specific to ASC but also found in the relatives of probands with specific language impairment, indicating overlap in symptomatology and potentially genetic aetiology (Ruser et al. 2007). Other studies finding significantly higher frequencies of communication/ pragmatic abnormalities in the biological relatives of autistic probands include Bolton et al. (1994) and Szatmari et al. (2000), using the Autism Family History Interview (FHI; Bolton et al., 1994), and Hurley et al. (2007) using a measure designed to

detect the BAP in parents of children with ASC (the Broad Autism Phenotype Questionnaire [BAPQ]; Hurley et al., 2007). Therefore, difficulties in the social use of language could be a reliable feature of the BAP. However, not all studies have found clear differences in the language and communication abilities of ASC relatives compared to clinical and typically developing controls. For instance, Pilowsky et al. (2003) found no differences in language difficulties (including scores on the PRS) between siblings of children with ASC and two clinical control groups; the siblings of children with developmental language disorder and the siblings of childen with learning difficulties. Similarly, Folstein et al. (1999) found no differences in language-related difficulties between the siblings of autistic probands and Down Syndrome probands, using the FHI. The researchers found a difference in PRS scores only when family members were split up into those with and without early language-related cognitive difficulties (as reported retrospectively by the parents).

ASC symptomatology in the language and communication domain of impairment can also include a significant delay in the acquisition, comprehension and articulation of speech. Subsets of autistic probands never acquire fluent speech, whilst others can speak spontaneously but have problems with the structural aspects of language (Tager-Flusberg and Joseph, 2003). It is not clear whether these difficulties are consistently found in the relatives of autistic probands. Language delay was reported in 22% of siblings of autistic probands between 2 and 6 years of age in a study by Chuthapisith et al. (2007) and 20% of siblings of children with ASC in a study by Constantino et al. (2010), half of which were also considered to exhibit 'autistic speech'. Likewise, delayed language

development was reported in a longitudinal study of younger siblings of children with ASC, aged 5 to 18 months (Iverson and Wozniak, 2007). Videotapes of ASC siblings at home with their caregivers revealed delays on communicative milestones including reduplicated babble and first words, as well as delays in language comprehension and expression. This was coupled with delays in the siblings' motor development (e.g. less time spent in different postures) suggesting a possible relationship between the early disruption of the motor and vocal systems during development which could play a causal role in ASC and the BAP. However, Iverson and Wozniak did not measure the siblings' general cognitive development so it is not clear whether they were showing signs of general developmental delay or specific delays characteristic of ASC and the BAP. Likewise, Stone et al. (2007) also reported poorer scores on a parental measure of language and communication called the MacArthur Communicative Development Inventories (Fenson et al., 1993) in the infant siblings of children with ASC versus a sample of typically developing children.

Other studies have examined language difficulties in older siblings of autistic probands. For instance Folstein and Rutter's seminal studies in 1977 found high concordance rates in MZ twin pairs (relative to DZ twin pairs) for broader autistic-related traits including articulation disorder and retrospective reports of language delay; 9 out of 11 non-autistic children in MZ pairs had cognitive/ language difficulties (82% concordance) compared to 1 out of 10 non-autistic children in DZ pairs (10% concordance). Support for the presence of similar characteristics in the relatives of autistic probands has also been described by Bolton et al. (1994) who reported broad language and communication

deficits using the FHI, including delays in the onset of speech and articulation difficulties. Bolton and colleagues also found a marked increase in the reporting of reading and spelling problems. Likewise in a study by Folstein et al. (1999), significantly more parents of children with ASC reported language-related difficulties including reading and spelling compared to parents of Down Syndrome children, although this was not found for siblings of autistic probands. When reading and spelling performance has been assessed in ASC relatives, differences in test scores have not been consistently found compared to control groups (e.g. Pilowsky et al., 2007; Schmidt et al., 2008; see section 1.3.2.4). Finally Landa et al. (1991) found significant differences between parents of autistic probands and parents of Down Syndrome probands on a measure of spontaneous narrative discourse. Overall, the current consensus indicates that language delay, social-pragmatic problems and spontaneous narrative discourse could be potential components of the BAP, with moderate support for both the structural components of language and reading, spelling and articulation difficulties.

1.3.1.2 Reciprocal Social Interaction

Significant impairment in reciprocal social interaction is a defining clinical feature of ASC and the literature currently suggests that a milder version of these behavioural impairments extends to the relatives of autistic probands. A large number of recent studies have examined social behavioural deficits in the at-risk infant siblings of children with an autism diagnosis. For example, at-risk siblings are less likely to respond to their name on the first or second call compared to typically developing children at 12 months

of age (Nadig et al., 2007). Infant siblings of autistic probands have also been reported to initiate joint attention significantly less frequently than a typically developing control group (e.g. Cassel et al., 2007; Goldberg et al., 2005; Landa et al., 2007). Similarly, siblings are less able at *responding* to joint attention compared to typically developing controls (Presmanes et al., 2007; but see Goldberg et al., 2005 for negative findings using a less sensitive measure of joint attention). Siblings later classified as 'BAP+' also displayed deficits responding to joint attention compared to siblings later classified as 'BAP-' (Sullivan et al., 2007). Other social behavioural deficits detected in at-risk siblings include reduced frequency of requesting behaviours (Goldberg et al., 2005; Cassel et al., 2007), reduced response to social interaction (Goldberg et al., 2005) and differences in eye gaze movements; for example, shifting gaze to and from the caregiver less frequently (Ibanez et al., 2008), gazing away from the caregiver for longer periods (Ibanez et al., 2008), gazing less at the caregiver's eyes relative to the mouth (Merin et al., 2007) and looking less at the caregiver and more at a novel object during a social-object learning task (Bhat et al., 2010). However, it is important to note that in a number of these studies there was no longitudinal follow-up to determine whether the infants that performed poorly on these tasks would express BAP traits later in development (e.g. Bhat et al., 2010; Goldberg et al., 2005; Merin et al., 2007; Nadig et al., 2007; Presmanes et al., 2007; Cassel et al., 2007). Instead the infants examined in these studies may later display the full ASC phenotype. Other studies have circumvented this problem by later classifying siblings into 'BAP+', 'BAP-' and 'ASD' groups (e.g. Landa et al., 2007 and Sullivan et al., 2007).

A small number of studies have examined socioemotional behaviour in at-risk ASC siblings during play with their caregivers. Using a paradigm called the 'face-to-face/ still face' (FFSF) task (Tronick et al., 1978), caregivers play with their child and are then asked to hold a still, expressionless face for a sustained period to increase negative emotion (cry-faces) and reduce positive emotion (smiling) in the infant, before the caregiver resumes play. Cassel et al. (2007) carried out a longitudinal study examining changes in positive and negative emotion generated by the FFSF task in infants at a low risk and high risk for ASC. They found that at 6 months, the siblings of children with autism smiled significantly less during the FFSF task than low-risk, typically developing infants. Likewise, Yirmiya et al. (2006) reported that infant siblings of children with autism got less upset and displayed more neutral affect during the still face procedure of the FFSF task. Those siblings that displayed higher rates of neutral affect during the still face procedure initiated fewer joint attention bids and requesting behaviours at 14 months. Also, mother-infant synchrony was poorer for infant-led interactions during free play in the ASC sibling group, compared to typically developing infant controls. The FFSF task has also been used to investigate eye gazing/ visual attention, with various studies reporting differences in eye gaze movements towards the caregiver and inanimate objects between at-risk siblings and low-risk, typically developing controls (e.g. Ibanez et al., 2008; Bhat et al., 2010; Merin et al., 2007). These studies suggest that differences in eye gaze movements could be an early indicator of the BAP.

Other studies looking at the early social BAP include Toth et al. (2007) and Christensen et al. (2010) who examined play behaviour in at-risk siblings. Using the Communication

and Symbolic Behavior Scale-Developmental Profile (Wetherby and Prizant, 2002), Toth et al. reported that infant siblings of children with ASC displayed less symbolic behaviour as well as fewer responsive social smiles and distal gestures such as pointing during social interactions. In contrast, using their own assessment of play behaviour, Christensen et al. reported no differences in the rates of symbolic play actions between a sample of at-risk siblings and typically developing infant controls at 18 months, although at-risk siblings showed significantly more non-functional repeated play behaviours than controls (see section 1.3.1.3).

A number of studies have suggested that difficulties in this domain extend to the adult relatives of autistic probands. Using a structured clinical interview, Wolff et al. (1988) reported that the parents of children with ASC displayed a greater lack of rapport and higher 'social gaucheness' compared to the parents of children with special needs (excluding ASC), whilst Gillberg (1989) found some qualitative evidence of mild social deficits in the parents of probands with Asperger Syndrome, based on interviews about family psychiatric history. Likewise, using a semi-structured interview, Narayan et al. (1990) described some parents of children with ASC as displaying social gaucheness. High rates of broadly defined social difficulties in first-degree relatives have also been reported by Bolton et al. (1994) and occasionally in second-degree relatives (grandparents, aunts and uncles) using the FHI (Piven et al., 1997a), which suggests that these problems could have a strong genetic liability. More recently, Szatmari et al. (2008) have suggested that alexithymia could be an important feature of the BAP: that is, a difficulty in identifying, describing and processing one's own emotions. Parents of

children with ASC scored higher than a clinical control group (the parents of children with Prader Willi Syndrome) on a self report questionnaire called the Toronto Alexithymia Scale (Bagby et al., 1994), especially on the subscale: 'difficulty identifying feelings'. In fathers, high alexithymia scores were associated with high levels of repetitive behavioural symptoms in their children with ASC, as measured using the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994).

Compared to both clinical and non-clinical control groups, the parents of children with ASC have been reported as having lower quality or quantity of friendships and a preference for less social activities and behaviours (e.g. Briskman et al., 2001; Losh and Piven, 2007; Losh et al., 2008; Piven et al., 1997a; Santangelo and Folstein, 1995). Some studies indicate gender differences in the degree of social impairment e.g. using the FHI, Piven et al. (1997a) reported that 57% of fathers of children with ASC had broadly defined social deficits compared to 13% of fathers of children with Down syndrome. This contrasted with 36% and 13% of mothers with ASC and Down syndrome respectively, suggesting that social impairments may be especially prevalent in male relatives of individuals with ASC. Similarly, using a new interview-based measure called the Broader Phenotype Autism Symptom Scale (Dawson et al., 2007), fathers of children with autism scored significantly higher than mothers on 2 domains including 'social expressiveness' (Dawson et al., 2007). Sex differences were also reported in a study by Virkud et al. (2009) who found significantly higher aggregations of autistic traits in the brothers of children with ASC using the Social Responsiveness Scale. However, rather than concentrating on unaffected relatives only, Virkud et al. included siblings with ASC

diagnoses in their analyses which elevated mean scores on this measure (see Hoekstra and Wheelwright, 2010). Future analyses of the BAP conducted by the same research group were modified to include unaffected relatives only, producing similar results: there was an aggregation of autistic traits in the unaffected relatives of siblings, especially brothers from multiple-incidence autism families (Constantino et al., 2010). This supported previous work carried out by the same research group reporting significantly reduced social responsiveness in the siblings of autistic probands compared to a clinical control group (Constantino et al., 2006). Research studies have also reported elevated scores on the 'social skills' subscale of the Autism-Spectrum Quotient in the parents of children with ASC compared to parents of typically developing children; this was especially true for fathers (Wheelwright et al., 2010; Ruta et al., 2011). Likewise, using the Communication Checklist-Adult Version, parents of children with ASC reported significantly higher scores on the subscale 'social engagement' (i.e. indicating greater deficits) compared to a large sample of typical adults from the general population (Whitehouse et al., 2010). Altogether, these studies indicate significant impairments in reciprocal social interaction amongst the relatives of autistic probands, particularly fathers and brothers, and provide evidence to warrant the inclusion of these behavioural traits in the BAP.

1.3.1.3 Repetitive, Stereotyped Behaviour and Interests

The third domain of symptoms characterising clinical diagnoses of ASC involve restricted, repetitive and stereotyped patterns of behaviour, interests and activities (DSM-IV-TR). To date, a modest number of studies have suggested that the relatives of autistic probands display a milder version of these clinical manifestations. In a study on infant siblings of children with ASC, Christensen et al. (2010) reported significantly higher frequency of non-functional repeated play behaviours compared to typically developing infants. In a study involving older relatives, Smith et al. (2009) carried out a factor analysis on the restricted, repetitive behaviours and interests (RRBI) domain of ASC using the Autism Diagnostic Interview-Revised and examined associations between RRBI and personality traits linked to ASC in the parents. They found that the factor 'intense preoccupations' in affected children correlated significantly with the personality traits 'rigid' and 'aloof' in fathers, suggesting that there may be a genetic association between these traits. The parents of children with ASC have also been reported as rigid/ perfectionistic in a small number of other studies (e.g. Losh et al., 2008; Piven et al., 1997b; see section 1.3.4). Wolff et al. (1988) interviewed parents of autistic probands and non-autistic children with special needs and found parents, and especially fathers, of children with ASC to exhibit special interest patterns (corresponding with the restrictive behaviours commonly found in autistic probands). However, this trait failed to distinguish parents of children with ASC from parents of non-autistic children with special needs. Likewise, Narayan et al. (1990) interviewed 21 parents of children with ASC and reported a significant tendency for parents to display a 'single-minded pursuit

of special, often intellectual, interests'. Bolton et al. (1994) found elevated rates of stereotyped behaviours in first-degree relatives of autistic probands compared to the relatives of Down Syndrome probands, whilst Piven et al. (1997a) reported similar findings in first and second-degree relatives of autistic probands, using the FHI; 26% of ASC fathers had stereotyped behaviours compared to 3% of Down Syndrome fathers whilst 12% of ASC mothers had stereotyped behaviours versus 0% of Down Syndrome mothers. Finally, parents of children with ASC were reported to score significantly higher than a clinical and non-clinical control group on an experimental questionnaire designed to tap into real-life non-social skills and preferences (e.g. insistence on routines and circumscribed hobbies; Briskman et al., 2001).

Overall, the small numbers of studies that have examined restrictive repetitive behaviours in first degree relatives of autistic probands have found some evidence of a BAP in this domain. This includes broadly defined stereotyped behaviours using the Autism Family History Interview, reports of real-life non-social skills and preferences and a rigid/ 'perfectionistic' personality. The studies that have so far examined this behavioural domain in ASC relatives have largely relied on categorical data. Future work should investigate repetitive, stereotyped behaviour and interests using quantitative, dimensional measures which are more sensitive to picking up subtle differences indicative of the BAP.

1.3.2 Cognitive level

1.3.2.1 Social Cognition

A wealth of research studies support the theoretical construct that people diagnosed with ASC have a significantly reduced ability to process information relating to other people's mental states, commonly referred to as a Theory of Mind (ToM; e.g. Baron-Cohen et al., 1997; Baron-Cohen et al., 2001a; Happé, 1994; White et al., 2009). These deficits in social cognition appear to be a key component of clinical ASC, although they are not necessarily universal to people with ASC, or specific to this disorder (Pellicano, 2011). Early studies suggested that ToM deficits were not part of the BAP e.g. Ozonoff et al. (1993) found no differences in performance on a second-order belief attribution task and two other ToM tasks between the siblings of children with ASC and two clinical control groups. However, sample sizes were small and measures may not have been sufficiently sensitive to pick up subtle differences indicative of the BAP. Later studies have generally found that relatives of autistic probands score significantly lower on specific performance measures of social cognition ability. A very well replicated finding is that relatives of people with ASC tend to perform poorly on the 'Reading the Mind in the Eyes' Test (Mind in Eyes; Baron-Cohen et al., 2001a) where participants have to identify complex psychological states from looking at pictures of the eye region of people's faces (Baron-Cohen and Hammer, 1997; Dorris et al., 2004; Losh and Piven, 2007; but see Gokcen et al., 2009). These studies collectively suggest that older relatives of autistic probands can experience mild difficulties on ToM tasks. Few studies have examined ToM ability in younger siblings of children with ASC. Shaked et al. (2006) tested siblings aged 54-57

months on two measures of ToM: the false belief task and the three easiest stories from the 'Strange Stories' task (Happé, 1994). No differences were found between siblings of children with ASC and a typically developing control group, but the measures used may not have been sufficiently sensitive to detect subtle ToM difficulties in siblings.

Social cognitive difficulties appear not to be restricted to advanced ToM tasks such as the Mind in Eyes test, but are also reported for tests of basic emotion recognition. For example, Palermo et al. (2006) asked parents of autistic probands to identify schematic facial patterns representing five 'basic' emotions, including happiness, anger, sadness, surprise and disgust. In identifying facial displays representing sadness and disgust, fathers of autistic probands performed worse than mothers of autistic probands. Both parents performed less well on average than controls, suggesting that difficulties understanding facial expressions extend beyond the clinical boundaries of ASC to include relatives of autistic probands. Likewise, Wallace et al. (2010) reported significantly reduced performance on a test of basic facial emotion recognition in parents and siblings of children with ASC from multiple-incidence autism families; relatives were significantly worse at identifying expressions of fear and disgust compared to typical controls from the general population. Similarly, a study by Bölte and Poustka (2003) detected poorer performance in the recognition of facial affect in the first-degree relatives of individuals with ASC from multiple-affected families compared to single-affected families. However, Bölte and Poustka found no significant differences overall between ASC parents and controls. Altogether, most recent studies support earlier findings in

smaller samples of ASC relatives, which described difficulties recognising emotions (Smalley and Asarnow, 1990).

Other important studies on the BAP that examine social cognition include Losh et al. (2009), where 38 probands with ASC, 83 parents of a child with ASC and a control group were examined using a variety of neuropsychological tests assessing participants' social cognition, executive functioning and central coherence (see later). Parents were divided into discrete 'BAP +' and 'BAP -' groups based on the presence or absence of rigid/ perfectionistic personality traits using an interview measure called the Modified Personality Assessment Schedule, Revised (Piven et al., 1994). Autistic probands and parents who were 'BAP +' were found to differ from controls on just one set of measures; those involving social cognition. These measures included the Mind in Eyes task, a task assessing people's trustworthiness of faces and a 'Movie Stills' task that assesses people's reliance on facial information to discern the emotional content of complex scenes.

These studies collectively suggest that a subset of the relatives of autistic probands struggle to recognise or represent other people's thoughts and emotions. However, despite these findings, it is still unclear whether poorer performance on ToM tasks represents a categorical entity of the BAP that is present in a subset of relatives *or* a set of continuously distributed traits that are significantly lower than population averages.

A small number of social cognition studies suggest that face processing strategy might be a component of the BAP. Adolphs et al. (2008) used a specially-devised 'bubbles' method (Gosselin and Schyns, 2001) to hide particular regions of the face during an emotion recognition task. Participants had to identify whether facial stimuli were 'happy' or 'sad' using information from specific features of the face. Parents of children with ASC classified as socially aloof ('BAP+') performed at near-identical accuracy on the task compared to parents of children with ASC who were not classified as socially aloof (BAP-'). However, the 'BAP+' group displayed reduced processing of information from the eye region of the face and enhanced processing of the mouth, relative to the 'BAP-' group.

Other studies investigating social cognition in ASC relatives suggest that face memory and face recognition could be components of the BAP. Parents of children with ASC were significantly impaired on the Cambridge Face Memory Test (Duchaine & Nakayama, 2006) compared to parents of typically developing children, whilst significant parent-proband correlations were found for a face matching task, suggesting that face recognition is heritable (Wilson et al., 2010). Given the large variability in performance on particular social cognition tasks by individuals on the autism spectrum, Wilson et al. stress that finding correlations within particular families can be as informative as finding significant differences between controls and experimental groups such as individuals with ASC and their first-degree relatives. A study by Wallace et al. (2010) also suggests that impaired face recognition is part of the BAP; the relatives of children with ASC from multiple-incidence autism families were less successful at discriminating subtle

differences between digitally altered pictures of faces compared to a control group from the general population. Difficulties appeared to relate specifically to social stimuli since relatives did not show similar difficulties discriminating differences between objects (pictures of houses). Despite these positive findings, significant differences between ASC relatives and control groups have not always been found on tests of facial recognition (e.g. Palermo et al., 2006 and Wilson et al., 2010).

Finally, there is evidence to suggest that relatives of autistic probands experience comparable but milder problems processing eye gaze. Wallace et al. (2010) reported differences between ASC relatives and controls on a directional judgement task examining eye gaze processing. Participants had to judge the direction of social (eye gaze) and non-social (arrow) cues which were presented on a screen for very short time durations. Relatives of children with ASC did not show an accuracy advantage for detecting direct compared to averted gaze, whilst controls did. ASC relatives therefore appear less sensitive to direct eye gaze than controls from the general population. Furthermore, problems using eye gaze to orient towards targets have been reported by Scheeren and Stauder (2008). Using a similar directional judgement paradigm involving the detection of targets using social (eyes) and non-social cues (arrows), Scheeren and colleagues found that fathers of autistic probands responded slower on social cues than control fathers (see section 1.3.2.3).

In summary, studies currently provide strong support for the inclusion of social cognitive traits in the later BAP. These include problems recognising basic facial expressions of emotion, higher order ToM difficulties (e.g. reading the mind in the eyes), mild problems processing people's eye gaze and possibly mild difficulties discriminating/ remembering faces (see Table 1.2). These different social cognitive features have been united together under the broader psychological construct of empathy (Baron-Cohen, 2002), and so it can be persuasively argued that there is strong support for a BAP for empathy-related difficulties. However, more studies are needed explicitly exploring this construct in older ASC relatives. In contrast, less support has been found for social cognitive deficits in young siblings of children with ASC (e.g. Shaked et al., 2006), although more research needs to be conducted on this experimental group examining social cognitive abilities.

1.3.2.2 Executive Function

Executive function is an umbrella term describing a collective set of functions such as planning, working memory, impulse control, inhibition, mental flexibility and the initiation/ monitoring of actions (Hill, 2004). Executive dysfunction is frequently cited as a leading theoretical construct purporting to explain ASC symptomatology (e.g. Ozonoff et al., 1993). Do the relatives of autistic probands show milder manifestations of executive functioning problems? Studies assessing executive function in the relatives of people with ASC have generated mixed findings. For example, Bölte and Poustka (2006) found no differences in test scores of executive function between parents of individuals with ASC and parents of individuals with early onset schizophrenia or intellectual disability; experimental and control groups were matched for age and non-verbal IQ. The

executive function tests used included: (1) the Wisconsin Card Sorting Test (Heaton et al., 1993), which measures a person's ability to flexibly shift cognitive strategies, form abstract concepts and respond to changes in the environment using feedback (2) the Tower of Hanoi Test (Simon, 1975), which measures higher order planning abilities and (3) the Trail Making Test (Reitan, 1979), which measures a person's speed and accuracy of attention and capacity to shift strategies in response to changes in the environment. Likewise, Losh et al. (2009) reported no significant differences on the Tower of Hanoi and Trail Making Test between BAP parents/ probands and controls and Pilowsky et al. (2007) found no differences in performance on the Tower of Hanoi and Word Associations Test (Semel et al., 1995) between ASC siblings and two clinical control groups (siblings of children with learning disabilities and developmental language delay). These studies contrast with early findings by Ozonoff et al. (1993) who reported significant differences in performance on the Tower of Hanoi between the siblings of children with ASC and two clinical control groups. Similarly, Hughes et al. (1999) reported that a greater number of ASC siblings performed poorly (compared to a clinical and non-clinical control group) on three executive function tasks from the Cambridge Neuropsychological Test Automated Battery (Robbins et al., 1994), including the Intra-Dimensional/ Extra-Dimensional Set Shifting Task (measuring attentional flexibility) and the Tower of London (measuring planning ability; Shallice, 1982). Likewise, studies by Delorme et al. (2007) and Nydén et al. (2011) found impairments in planning ability, based on poorer performance on the Tower of London by the unaffected siblings and parents of children with ASC compared to a control group from the general population. However, poorer performance on the Tower of London (relative to healthy controls) was

also found in the relatives of children diagnosed with Obsessive Compulsive Disorder, so impaired planning ability may not relate specifically to the relatives of autistic probands (Delorme et al., 2007). Other reports of significantly reduced planning capacities in older relatives of autistic probands compared to control groups, include Piven and Palmer (1997) (lower test scores on the Tower of Hanoi) and Hughes et al. (1997) (lower test scores on the Tower of London). However, neither study matched parent groups for non-verbal IQ; the former found significant differences between groups on non-verbal (performance) IQ whilst the latter matched parent groups by child IQ and age. In contrast, Wong et al. (2006) did not find significant reductions in planning and inhibition amongst ASC relatives, when matched with a control group for chronological age, performance IQ and verbal IQ, but instead found poorer performance on a test of generativity (ideational fluency). Given that generativity problems have also been reported for autistic probands (e.g. Dichter et al., 2009), it is possible that these impairments may be genetically associated with ASC. However, these studies contrast with others that provide mixed or negative support for other kinds of generativity tasks such as verbal/ design fluency (e.g. Delorme et al., 2007; Pilowsky et al., 2007; Schmidt et al., 2008).

Other recent positive results on executive functioning tasks include a study by Sumiyoshi et al. (2010) who reported similarities in performance by individuals with ASC and their siblings on the Wisconsin Card Sorting Test and a test of working memory; the Verbal Learning Task (Gold et al., 1992). Compared to a control group, both individuals with ASC and their siblings recorded an elevated rate of perseverative errors on the Wisconsin Card Sorting Test and displayed a diminished ability to record the number of exemplars in the same category during the Verbal Learning Task. Experimental and control groups

were matched by age but there were significant differences in IQ amongst the groups, meaning that the differences found could have been due to general cognitive ability differences rather than a selective impairment in executive functioning.

Other studies examining executive functioning processes have focused on working memory. Koczat et al. (2002) reported spatial working memory deficits during a delayed oculomotor task in the parents of autistic probands. However, some studies support superiorities on the spatial span task, which assesses visuospatial working memory (e.g. Hughes et al., 1999; Mosconi et al., 2010) These findings contrast with others in older relatives that have found no differences on working memory tasks (e.g. Hughes et al., 1997, 1999; Wong et al., 2006). In younger relatives, a study by Noland et al. (2010) found *enhanced* working memory for non-social targets in at-risk infant siblings of children using a delayed-response task. Taken together, the results of studies examining working memory in ASC relatives are inconsistent.

In summary, the findings from BAP studies focusing on executive functioning have been mixed, and differences between relatives of people with ASC and controls tend to diminish when groups are matched for general cognitive ability. Moreover, executive functioning difficulties are not specific to ASC but can be found in a number of psychiatric conditions, such as attention deficit/ hyperactivity disorder and schizophrenia (e.g. Bölte and Poustka, 2006). Therefore, whilst executive function problems may be part of the BAP, their low specificity needs to be taken into account when deciding whether such problems indicate a specific genetic liability for autistic traits in relatives. In addition, the executive function tasks may not be efficiently tapping into specific, unitary cognitive processes and so better measures are needed to determine which

cognitive operations might be disrupted in ASC and the BAP (see Ozonoff et al., 1993). With this caveat in mind, the best supported prospective BAP traits in this cognitive domain include superior performance on the spatial span task and higher level planning deficits. There is also early support for ideational fluency difficulties (see Table 1.2). However, in general studies investigating executive functioning processes have yielded mixed results so it is not clear whether any component of this cognitive domain is a definitive feature of the BAP.

1.3.2.3 Visual attention, sensory integration and sensorimotor functioning

Some studies have found significant differences in visual perception or attention in autistic probands compared to control groups (e.g. Jolliffe and Baron-Cohen, 1997; Shah and Frith, 1983). This is hypothesised to reflect a different 'cognitive style' that leads to superior performance on tests where local visual processing is an advantage, including the Embedded Figures Task (EFT; Witkin et al., 1971; e.g. Grinter et al., 2009; Jolliffe and Baron-Cohen, 1997; Shah and Frith, 1983; but see White and Saldaña, 2011) and the Block Design Task (BDT; Weschler, 1949; Shah and Frith, 1993). There is evidence to suggest that a similar local processing style is manifested to a lesser extent in first-degree relatives, for example, Baron-Cohen and Hammer (1997) and Bölte and Poustka (2006) reported significantly faster times on the EFT in the parents of autistic probands compared to controls, indicating a similar tendency towards local visual processing. Superior performance on the EFT by fathers of autistic probands was also reported by Happé et al. (2001) together with a reduced susceptibility to visual illusions, perhaps reflecting important differences in visual processing and attention. Other studies reporting superiorities in visuospatial abilities in ASC relatives include Smalley and

Asarnow (1990), where siblings of autistic probands performed above average on the BDT and the Benton Test of Line Orientation (Benton et al., 1975). Despite these positive findings, there have been a number of studies that have failed to find support for a local processing style in the relatives of autistic probands, especially the BDT (Bölte and Poustka, 2006; Fombonne et al., 1997; Losh et al., 2009; Piven and Palmer, 1997; Scheeren and Stauder, 2008) but also the EFT (e.g. Losh et al., 2009). This mirrors problems replicating a local processing style across tasks and domains in clinical cases of ASC (see White and Saldaña, 2011).

Whilst a number of studies on autistic probands and their relatives have found superior performance on tasks requiring strong attention to detail, studies assessing divided attention indicate possible impairments in people with ASC and their relatives. In a study by Belmonte et al. (2010), participants had to simultaneously attend to spatially disjoint, non-social stimuli and suppress intervening distractive information. Therefore, the task required a 'complex' form of processing that involved rapidly processing and integrating information from multiple inputs (in this instance, requiring selective attention to colour and orientation of stimuli in disjoint, peripheral locations). Results showed that the ASC group performed worst on the divided attention task, followed by the siblings of the probands followed by age and IQ-matched controls. This finding suggests that divided attention problems could be a reliable candidate trait for the BAP.

As well as difficulties attending to different stimuli at the same time, relatives of autistic probands may also experience problems shifting attention. A study by Scheeren and Stauder (2008) suggests that fathers of children with ASC exhibit disturbances in the engagement of attention. This conclusion was based on differences in time patterns on a

reaction time task which examined shifts of attention in response to social and non-social cues. Visual attention patterns have also been examined in younger infant siblings of children with ASC as an early indicator of the BAP (see also section 1.3.1.2). The results of current studies are slightly mixed but there is some evidence that siblings who are at-risk for ASC display early problems disengaging from stimuli and spend longer periods attending to non-social stimuli (e.g. see Ibanez et al., 2008 and Bhat et al., 2010). Similar findings were reported by Elsabbagh et al. (2009b) who tested 9-10 month old siblings of autistic probands using a visual orienting task that measured the time taken to disengage from a central stimulus in order to fixate on a peripheral one. Infant siblings of autistic probands exhibited longer disengagement latencies compared to a control group, indicating problems with the early-developing ability to switch attention flexibly. ASC siblings were also worse at automatically orienting to visual targets and forming expectations about their visual environment. A study by Holmboe et al. (2010) did not find significant group differences in attentional disengagement between at-risk siblings and typically developing controls on a task of inhibitory control (the Freeze-Frame task; Holmboe et al. 2008). However, significantly more infants in the ASC sibling group had problems disengaging from a central stimulus compared to the control group within a subset of infants showing sticky fixation. Therefore, problems in visual orientation, particularly attentional engagement and disengagement, are strong contenders for inclusion in the BAP. Additionally, the finding that ASC siblings spend significantly longer looking at their caregiver's mouth and less time at the eyes compared to typically developing controls (Merin et al. 2007; see section 1.3.1.2) is suggestive of problems in visually attending to the most informative features of social stimuli.

Finally, a study by Mosconi et al. (2010) has detected oculomotor abnormalities in the first-degree relatives of individuals with ASC. Using tests of sensorimotor responses to visual stimuli, relatives displayed saccadic dysmetria and increased variability of saccade accuracy. They also displayed left-lateralised deficits in smooth-pursuit eye movement (open-loop pursuit gain) and procedural learning for rightward saccades. Some of these results have also been found in samples of individuals with ASC (e.g. Takarae et al., 2004) suggesting that alterations in the neural circuitry recruited for these tasks is a heritable component of ASC and a candidate feature of the BAP. Other studies examining oculomotor functioning in first degree relatives of autistic probands include Koczat et al. (2002). Parents of children with ASC were found to show significantly poorer spatial accuracy on a delayed oculomotor response task designed to detect spatial working memory deficits compared to a sample of adult controls.

Studies therefore broadly provide support for visual attention difficulties in the first degree relatives of autistic probands, especially attentional engagement/ disengagement, divided attention and oculomotor abnormalities, with mixed findings for local visual attention biases. However, further research is needed replicating studies that report significant differences between ASC relatives and controls in this cognitive domain. Future studies should also more broadly focus on the psychological constructs that help explain cognitive superiorities of ASC in unaffected relatives of autistic probands, such as examining their tendency to 'systemise', which is the drive to construct and analyse the variables within a system (Baron-Cohen et al., 2003). Finally, future research could also examine other sensory modalities and investigate associations between the BAP and elevated sensory hypersensitivity. Some studies suggest that autistic probands detect

sensory stimuli at lower thresholds (Baron-Cohen et al., 2009). It remains to be explored whether this phenomenon can also be observed (perhaps to a lesser extent) in unaffected relatives of individuals with ASC.

1.3.2.4 Language Ability

To complement the investigation of language impairments in the relatives of individuals with ASC using questionnaires and interviews (see 'Language and Communication'), researchers have administered a number of performance measures of language ability. A study by Schmidt et al. (2008) investigated phonological processing in ASC parents using the non-word repetition task (Gathercole and Baddeley, 1990). Schmidt reported poorer performance on this task compared to adult controls suggesting that phonological processing deficits could be a component of the BAP. Also, a study by Lindgren et al. (2009) investigated expressive language, lexical comprehension and phonological processing in people with ASC, specific language impairment and their first-degree relatives. Relatives of autistic probands were superior on tests of non-word repetition/ phonological processing compared to relatives of probands with specific language impairment. Whilst relatives of children with ASC *and* language delay scored lower on measures of reading ability and receptive language than relatives of children with ASC *without* language delay, no statistically significant differences were found on measures of expressive language or phonological processing. Lindgren et al. concluded that phonological deficits were not part of the heritable phenotype of ASC, and so should not be included in the BAP.

A study by Losh et al. (2010) investigated Rapid Automatised Naming (RAN) ability in individuals with High Functioning Autism and their parents. Both groups exhibited significantly slower times on two rapid naming tasks (colour and object naming) compared to typically developing children and their parents. This supported a previous study that found significant differences between parents of children with ASC and controls on the same two subtests of the RAN task (Denckla and Rudel, 1974; Piven and Palmer, 1997). Furthermore, Losh et al. (2010) found significant associations between parents' times on these tasks and the social and language-behavioural features of the BAP, measured by the FHI and the Modified Personality Assessment Schedule. These features include a socially aloof/ untactful personality and retrospective reports of language delay. There was also a significant association between the RAN performance of fathers and their child with ASC, suggesting that this trait is heritable. However, not all studies have found significant differences between ASC relatives and controls on this measure (e.g. Pilowsky et al., 2003). It should be noted that whilst RAN tasks are an effective measure of expressive language ability, they also involve a number of neuropsychological domains including executive control and attentional processes. Therefore, whilst RAN is a candidate trait of the BAP and a potential indicator of liability to ASC, the measure does not have strong structural and functional specificity.

Performance measures that have examined receptive and expressive language ability have generally not found impairments in parents and non-infant siblings (e.g. Lindgren et al., 2009; Pilowsky et al., 2003; Schmidt et al., 2008). Studies focusing on the younger infant siblings of children with ASC have provided stronger support for milder expressive/ receptive language difficulties e.g. Gamliel et al. (2009) examined children between 14

and 54 months using a battery of language and general cognitive measures, reporting significant differences in language scores between typically developing controls and children later displaying the BAP at 7 years of age. Likewise, Toth et al. (2007) reported that 18-27 month old siblings of children with ASC had lower receptive language skills than typically developing controls as well as displaying below average expressive language ability, using the Mullen Scales of Early Learning (Mullen, 1997). However, using the Clinical Evaluation of Language Fundamentals, Levy and Bar-Yuda (2011) found no differences in language ability between infant ASC siblings and typically developing controls when IQ was controlled for. Using the same measure, Stone et al. (2007) found no differences in expressive language ability between 12-23 month year old ASC siblings and typically developing controls.

Finally, studies provide moderate support for poorer performance on tests of reading or spelling in the relatives of autistic probands, compared to controls (e.g. Fombonne et al., 1997 and Piven and Palmer, 1997). These studies contrast with others that have reported no differences (e.g. Freeman et al., 1989; Pilowsky et al., 2007; Whitehouse et al., 2007) or superior performance compared to other clinical groups (e.g. dyslexia; Happé et al., 2001).

Overall, studies provide moderate support for impairments in language ability, both in the early emerging BAP in infant siblings and the later BAP in older relatives. Prospective traits for the BAP include expressive or receptive language difficulties in infant siblings and impaired performance on the RAN task and poorer reading ability in older relatives. However in general the results of studies analysing language performance do not strongly substantiate the inclusion of these traits in the BAP.

1.3.2.5 Contrast Sensitivity/ Motion Perception

A very small number of research studies have examined contrast sensitivity and visual perception of motion in the relatives of autistic probands. Impaired visual motion perception has been reported in people diagnosed with ASC. At a neurological level, this has been linked to the atypical functioning of the subcortical magnocellular pathway that processes visual information. This can be tested by measuring participants' contrast sensitivity for luminance and chromatic light using sinusoidal gratings that are presented at different spatial and temporal frequencies. Contrast sensitivity can be measured both for the detection of a moving stimulus and for correctly discriminating the direction that the stimulus is moving. A study by Koh et al. (2010) detected inefficient motion processing for luminance stimuli in both people with ASC and unaffected siblings of individuals with ASC compared to typically developing adolescents. Furthermore, the study reported significantly higher chromatic contrast sensitivity in the adolescent siblings of autistic probands compared to typical controls. Chromatic contrast sensitivity in siblings was also higher than in autistic probands, leading Koh et al. to suggest that higher chromatic sensitivity could be a protective factor against full-scale ASC. A study by McCleery et al. (2007) also reported abnormal contrast sensitivity in the younger infant siblings of children with ASC, aged 6 months. Using the forced-choice preferential looking technique (Teller, 1979), at-risk siblings appeared to be twice as sensitive to luminance (light/ dark) stimuli than typically developing controls whilst exhibiting identical sensitivity to chromatic (red/green) stimuli. McCleery and colleagues inferred that these results indicated atypical functioning of the magnocellular visual pathway in the at-risk sibling group as well as their autistic relatives. These studies contrast with De

Jonge et al. (2007) who found no evidence for significant differences in contrast sensitivity, motion and form perception in both people with ASC and parents of people with ASC compared to a control group. Therefore, more research is required replicating studies examining contrast sensitivity and motion perception in ASC relatives. The above positive findings in this cognitive domain must also be placed in the wider context of studies examining motion perception in people diagnosed with ASC, which have yielded mixed results (e.g. De Jonge et al., 2007; Jones et al., 2011; Pellicano et al., 2005; Spencer et al., 2000).

1.3.2.6 General cognitive abilities

Intellectual disability (ID)[8] is common in autistic disorder, with a prevalence of approximately 70% in diagnosed cases (Fombonne, 2006). However, when the other conditions on the autism spectrum are also included (Asperger Syndrome and PDD-nos), the prevalence of ID in autism is considerably lower (e.g. Chakrabarti and Fombonne, 2005). The exact aetiological link between ASC and ID is unclear, with twin studies producing conflicting results (e.g. Hoekstra et al., 2009, 2010; Taniai et al., 2008).

Studies focusing on the relatives of people with ASC have generally found that ID is *not* a feature of the BAP. For example Fombonne et al. (1997) assessed the first-degree relatives of 99 ASC probands and 36 Down Syndrome controls on standardised tests of intellectual functioning and did not find an increased incidence of ID among ASC relatives. These results corroborated earlier findings by Freeman et al. (1989) and Szatmari et al. (1993) that found no mild cognitive deficits in the relatives of people with

[8] Intellectual Disability (previously referred to as mental retardation, DSM-IV) is most commonly defined by an IQ score equal to or below 70.

autism. A study by Starr et al. (2001) suggested that the liability of relatives of autistic probands to express the cognitive and social deficits associated with the BAP did not depend upon the IQ of the clinically diagnosed family member. This suggests that the BAP and general cognitive ability are largely independent of each other. Likewise, a study by Yirmiya et al. (2007) on infant siblings of children with ASC did not find delays in general mental development compared to siblings of typically developing children. Altogether, these studies point towards a limited genetic association between ID and ASC (Hoekstra et al., 2009) and suggest that general cognitive ability does not play a major role in the BAP.

1.3.3 Other Psychiatric Conditions

Studies into the BAP often show that whilst autistic probands and their relatives exhibit a number of atypicalities in different domains of functioning, similar impairments may be found in other psychiatric conditions such as: (1) executive dysfunction in schizophrenia and attention deficit/ hyperactivity disorder (e.g. Bölte and Poustka, 2006; see also Happé and Ronald, 2008), (2) ToM deficits in schizophrenia (e.g. Frith and Corcoran, 1996) and (3) communication difficulties in specific language impairment (e.g. Whitehouse et al., 2007). This suggests that there could be genetic or epigenetic overlap between different psychiatric conditions e.g. ASC and attention deficit/ hyperactivity disorder (Rommelse et al., 2011). Support for this view is provided by studies documenting the aggregation of other psychiatric disorders in ASC families (see Lainhart, 1999 for a review of early findings).

A number of studies have documented higher rates of affective disorder, depression, social phobia and anxiety in the relatives of autistic probands compared to control groups. Using family history and direct interviews, Piven and Palmer (1999) reported familial aggregation of other psychiatric conditions including social phobia and major depressive disorder compared to a clinical control group. Earlier studies carried out by Piven and colleagues had reported high rates of affective disorder and anxiety disorder in siblings and parents of children with ASC (Piven et al., 1990, 1991). Using the FHI, Bolton et al. (1998) found significantly higher rates of other psychiatric conditions in relatives of autistic probands compared to a clinical control group, including major depressive disorder. Although psychiatric conditions such as affective disorders rarely occurred together with the BAP, the high familial aggregation of these conditions suggests relatives of autistic probands have an increased susceptibility to a number of different psychiatric problems. Higher rates of depression in the first degree relatives of people with ASC have been reported in a range of studies, both when comparing the rates to general population (e.g. Gold, 1993; Micali et al., 2004) and clinical control samples (e.g. Smalley et al., 1995) Finally, a recent study by Ingersoll et al. (2011) reported increased depressed mood in mothers of children with ASC compared to mothers of typically developing children. Furthermore, depressed mood was predicted by a measure of the BAP (combined social-communication subscale of the AQ) after controlling for parenting stress and the severity of the child's ASC.

High rates of obsessive compulsive disorder have also been found in the relatives of autistic probands compared to control groups (Wilcox et al., 2003). Moreover, high numbers of obsessive-compulsive traits in parents have been linked to high scores in the

autistic proband on the repetitive behaviour domain of the ADI-R; correlations were strongest between fathers and child (Hollander et al., 2003). A study by Micali et al. (2004) on families with a child with a PDD found significantly higher rates of second-degree relatives with an obsessive compulsive disorder, whilst Bolton et al. (1998) reported higher rates of obsessive compulsive disorder in the first-degree relatives of autistic probands.

Altogether, these studies suggest that ASC relatives may be at an increased risk for developing other psychiatric conditions in comparison to both non-clinical and clinical control groups; particularly obsessive compulsive disorder, anxiety, social phobia and mood disorders such as depression. Many reports of clinical depression in the parents of children with ASC have an onset before the birth of the child with ASC (e.g. 75% of mothers reported by Micali et al., 2004). This suggests that increased rates of psychiatric conditions (such as anxiety and major depression) may have a genetic link with ASC and are not just caused by the stress associated with looking after children with clinical diagnoses; a meta-analysis of psychiatric disorders in parents of children with ASC by Yirmiya and Shaked (2005) seems to support this conclusion. Yirmiya and Shaked reported higher rates of other psychiatric conditions in the parents of children with ASC compared to parents of typically developing children or children with conditions that do not have a genetic liability (e.g. Down Syndrome). However, higher rates of psychiatric conditions were also found in groups carrying other known genetic liabilities, such as language/ learning disabilities, suggesting that the familiality of other psychiatric conditions is not an exclusive feature of ASC.

1.3.4 Personality Traits

The personality traits of relatives of autistic probands have been extensively studied by researchers and are frequently cited as components of the BAP. These are restricted to specific personality traits, which are believed to reflect an underlying genetic liability for ASC. The personality characteristics described more commonly in the relatives of autistic probands compared to relatives of typically developing children or children with another medical condition (e.g. Down Syndrome; Piven et al., 1997b) include 'rigid' (Hurley et al., 2007; Losh et al., 2008; Piven et al., 1997b; but see Murphy et al., 2000), 'impulsive' (Murphy et al., 2000) 'aloof' (Hurley et al., 2007; Losh et al., 2008; Piven et al., 1994, 1997b; Murphy et al., 2000), 'shy' (Murphy et al., 2000), 'tactless' (Piven et al., 1994; Losh et al., 2008; but see Murphy et al., 2000) 'reserved/ schizoid' (Bölte et al., 2007), 'irritable' (Murphy et al., 2000) 'hypersensitive to criticism' (Piven et al., 1997b) 'neurotic' (Losh et al., 2008), 'undemonstrative' (Piven et al., 1994; but see Murphy et al., 2000) and 'anxious' (Losh et al., 2008; Murphy et al., 2000; Piven et al., 1997b). A factor analysis carried out by Murphy et al. (2000) detected three clusters of personality traits that were more common in the relatives of autistic probands compared to relatives of Down syndrome probands; these were called 'withdrawn', 'difficult' and 'tense'. However, only the 'withdrawn' factor was significantly associated with the broader behavioural phenotype of ASC, which was measured using the FHI. These personality traits may also be related to performance on cognitive BAP measures (see Losh et al., 2009) as well as the core behavioural domains of ASC. A recent study by Seidman et al. (2011) reported sex differences in personality traits in fathers and mothers of children

with ASC. Using the BAPQ, fathers were rated by their respective partners as more 'aloof' than mothers, whilst mothers were rated by their respective partners as more 'rigid' than fathers. Seidman et al. note that the high ratings of 'rigidity' in mothers could be due to pressure to adapt to a rigid lifestyle in order to make their autistic child's environment more predictable and structured. Further research could investigate the relationship between the personality traits of ASC relatives and the increased risk to developing other psychiatric conditions (e.g. anxiety and depression), and the association between these traits and neuroanatomy and neurofunctionality. These latter topics will be the focus of the next paragraph.

1.3.5 Neuroanatomical and neurofunctional correlates of the BAP

1.3.5.1 Neuroimaging studies in ASC

A complementary level of analysis for understanding the aetiology of ASC is to examine potential neuroanatomical and neurofunctional correlates of autistic traits and to determine whether these correlates extend to the relatives of autistic probands. ASC has been linked to an acceleration of brain growth at around 12 months of age, with macrocephaly found in 15-20% of diagnosed children by 4-5 years of age (Minshew and Williams, 2007). Neuroimaging data provides evidence for abnormal growth in grey and white matter which are responsible for processing and transferring information between brain regions (Amaral et al., 2008; Courchesne et al., 2007; Schumann et al., 2010). In particular, there is atypical growth in the frontal and temporal lobes and in structures

within the limbic system such as the amygdala. These regions are heavily involved in social behaviour and communication (Amaral et al., 2008; Courchesne et al., 2007). Neuroimaging studies also show differences in patterns of activation, with information taking a longer time to be processed throughout the brain of individuals with ASC (Belmonte et al., 2010; Gepner and Féron, 2009). This is hypothesised to be a consequence of local over-connectivity and long-range underconnectivity between separate functional brain regions (Belmonte et al., 2004). A small number of studies have reported functional local over-connectivity in the brains of individuals with ASC during behavioural tasks (e.g. Schmitz et al., 2006). In contrast a large number of studies have detected long-range functional under-connectivity, such as Kleinhans et al. (2008) who found disconnections between the fusiform face area, left amygdala, posterior cingulate and thalamus during a face processing task (see Wass, 2011 for a review of connectivity studies). In general, brain imaging studies suggests there is less functional connectivity between brain regions linked to perception, social cognition, language and problem-solving in individuals with ASC (Belmonte et al., 2004; Courchesne et al., 2007; Minshew and Williams, 2007; Isler et al., 2010).

Have similar findings been reported in the relatives of autistic probands? A number of studies have examined functional differences in regions comprising the 'social brain', including the amygdala, superior temporal sulcus, fusiform face area, orbitofrontal cortex and anterior cingulate cortex (Brothers, 1990; Spencer et al., 2011). These are documented below, followed by studies examining other brain regions and behavioural

paradigms as well as studies examining neurostructural differences in ASC relatives. The main findings of these studies are summarised in Table 1.3.

1.3.5.2 ToM/ emotion recognition

A preliminary fMRI study on 12 parents of children with Asperger Syndrome by Baron-Cohen et al. (2006) indicated atypical brain activity during the Mind in Eyes task, relative to sex- and IQ-, but not age-, matched controls from the general population. There was reduced activity in the mid temporal gyrus and the inferior frontal gyrus during completion of the ToM task in the parents of autistic probands compared to gender-matched controls. Similarly, Spencer et al. (2011) reported significantly reduced fMRI activity in a group of siblings of autistic probands when responding to happy versus neutral faces during an emotion recognition task. Relative to an adolescent control group, attenuated activity was found in a variety of regions associated with socio-emotional functioning, including the Fusiform Face Area and the Superior Temporal Sulcus. Therefore fMRI response to happy faces could be a sensitive neuroimaging marker of the BAP.

Table 1.3: *Neurofunctional and neurostructural atypicalities linked to the aetiology of the BAP.*

Type of euroimaging study	Brain Region(s) affected	Functional or Structural Atypicality	Task	Relative(s) studied	Study
. fMRI	Left medial Temporal Gyrus, Inferior Frontal Gyrus	Hypoactive	The Mind in Eyes test	Parents	Baron-Cohen et al. (2006)
	Temporal Poles, right middle/ left posterior Superior Temporal Sulcus, right Fusiform Face Area, left superior Frontal Gyrus, left dorsomedial prefrontal cortex.	Hypoactive	Facial Emotion Processing Task (Happy vs. Neutral)	Siblings	Spencer et al. (2011)
	Fusiform Gyrus	Hypoactive	Facial Recognition task	Siblings	Dalton et al. (2007)
	Fusiform Gyrus, Left dorsolateral prefrontal cortex, Right inferior Temporal Gyrus	Hypoactive	Biological motion task	Siblings	Kaiser et al. (2010)
	Extra striate cortex: left lingual gyrus and right middle occipital gyrus	Hypoactive	The Embedded Figures task	Parents	Baron-Cohen et al. (2006)
	Fronto-cerebellar complex	Delayed activation	Visual 'divided attention' task	Siblings	Belmonte et al. (2010)
. Near-ıfrared pectroscopy	Anterior Prefrontal cortex	Changes in [oxy-Hb] intermediate between autism and controls	Verbal fluency task	Siblings	Kawakubo et al. (2009)
. ERP	Inferior right and left posterior temporal electrodes	Shorter latency N170 to faces vs. Objects/ No right-hemisphere lateralised ERP pattern to faces	Face recognition sub-tests from WMS-III and Woodcock Johnson Object Recognition Sub-test	Parents	Dawson et al. (2005)
	Anterior central, left and right temporal and posterior electrodes	Prolonged latency in 'P-400' ERP component in response to direct gaze	Direct vs. Averted Gaze Task using static face stimuli	'At-risk' infant siblings	Elsabaggh et al. (2009a)

Type of euroimaging study	Brain Region(s) affected	Functional or Structural Atypicality	Task	Relative(s) studied	Study
. MEG	N/A	Increased induced gamma-band power at 40Hz/ reduced evoked gamma-band power/ phase-locking factor	Presentation of auditory (pure-tone) stimuli	Parents	Rojas et al. (2008)
	N/A	Reduced gamma-band phase locking factor and phase-locked power	Presentation of auditory stimuli: 30/40/48 Hz amplitude-modulated sounds	Parents	Rojas et al. (2011)
. sMRI	Amygdala	Smaller volume	N/A	Siblings	Dalton et al. (2007)
	Left Hippocampus	Larger volume	N/A	Parents	Rojas et al. (2004)
	Inferior/ medial Frontal Gyri and cerebellum	Significant increases in gray matter	N/A	Parents	Peterson et al. (2006)
. DTI	Temporo-parietal junctions, medial prefrontal and superior temporal regions	Significantly reduced white matter/ axial diffusivity	N/A	Siblings	Barnea-Goraly et al. (2010)

1.3.5.3 Face Processing

Neurofunctional correlates of the BAP were also assessed using fMRI by Dalton et al. (2007) who detected significantly reduced levels of gaze fixation and brain function in the unaffected siblings of autistic probands compared to typically developing controls in response to a face-processing task. Using eye tracking techniques, both autistic probands and unaffected siblings were found to spend significantly less time fixating the eye region of the face compared to controls whilst viewing photographs of familiar and unfamiliar faces. Reduced brain function was reflected by decreased activity within the right hemisphere of the fusiform gyrus in both autistic probands and their unaffected relatives compared to controls. However, in siblings and controls there was a positive correlation between eye fixation and fusiform activation, suggesting that reduced activation in the right fusiform gyrus in siblings may be due to differences in how faces are scanned that have a 'downstream' effect on right fusiform activity, rather than there being a fundamental problem with the right fusiform gyrus per se.

1.3.5.4 Biological motion processing

Kaiser et al. (2010) found commonalities in brain activity between children with ASC and their siblings in response to a task assessing sensitivity to biological motion using point-light displays. Results implicated shared areas of atypical function in the left dorsolateral prefrontal cortex, the right inferior temporal gyrus and the bilateral fusiform gyrus. Importantly, siblings who exhibited subtle social and communication difficulties

were excluded. The authors suggest that at a neurological level, genetic relatives of individuals with ASC share subtle disruptions in brain function that are not necessarily picked up at a behavioural level. The authors further speculate that brain response to biological motion reflects a genetic vulnerability to ASC in relatives of individuals with ASC that may be compensated for during development by unique areas of activation in the ventromedial prefrontal cortex and right posterior superior temporal sulcus.

1.3.5.5 Visual Attention

Brain activity during a visual search task was investigated for 12 parents of children with Asperger Syndrome by Baron-Cohen et al. (2006). The results of fMRI scans indicated reduced activation of the right middle occipital gyrus and the left lingual gyrus during completion of the visual search task, relative to sex and IQ-matched controls. Likewise, fMRI was used by Belmonte et al. (2010) in a study assessing visual attention in autistic probands and clinically unaffected brothers. Both probands and brothers performed significantly less well on a visual divided-attention task (see section 1.3.2.3) which at a neurobiological level was detected by atypical fronto-cerebellar activation correlating with the psychometric measures of autistic traits. Results on the divided-attention task suggested that both ASC probands and, to a lesser degree, their siblings displayed atypical spatial distribution of visual attention. Neuroimaging data showed that in the ASC group, posterior cortices linked to lower-level processing were over-active and frontal cortices were under-active; in the ASC sibling group, differential activation between conditions was much more limited. The fronto-cerebellar attention systems were

activated in the autism and sib-autism group but were time-delayed, suggesting that it was the differential timing of activation that was causing poorer performance, rather than differences in activation *per se*. Despite showing a similar response to the ASC group, stronger activity was measured in the prefrontal brain regions of the unaffected sibling group. The authors suggest that the stronger activity may be a compensatory strategy for differences in neural processing that ensured connectivity was maintained between different brain regions recruited for the task.

1.3.5.6 Executive Function

Kawakubo et al. (2009) examined prefrontal cortex activation in the unaffected siblings of autistic probands during an executive functioning task (the letter fluency task). Kawakubo and colleagues examined brain activity by measuring changes in haemoglobin concentration in the prefrontal cortex using near-infrared spectroscopy. Siblings ranged in age from 5 to 39 years; in child siblings, there were no significant changes in haemoglobin concentration relative to controls but for adult siblings, increases in haemoglobin was intermediate between controls and adults with ASC, despite similar behavioural performance on the task across the three groups. Unaffected siblings showing evidence of the behavioural BAP with a questionnaire called the Childhood Autism Rating Scale-Tokyo Version (Kurita et al., 1989) were removed from analyses suggesting that neurofunctional measures were sensitive at detecting differences between first degree relatives and controls that are not picked up at a behavioural level.

1.3.5.7 ERP Studies and the BAP

In addition to using MRI to assess the neuroanatomy and neurofunctional correlates of the BAP, electrophysiological studies have provided further evidence for neurofunctional differences in relatives of autistic probands compared to controls. These include an event-related potential (ERP) study (Dawson et al., 2005) of face and object recognition in ASC parents and controls, focusing on an ERP component known as the N170 that preferentially activates to faces. Unlike controls, Dawson et al. reported that in parents of autistic probands there was an absence of right-hemisphere lateralised N170 ERP to faces. Furthermore, ASC parents, unlike controls, failed to show a faster N170 to faces compared to objects. These results mirror the pattern seen in individuals diagnosed with ASC (e.g. Dawson et al., 2002). Other studies using ERP include Elsabbagh et al. (2009a) which found that both autistic probands and their infant siblings had a slower 'P-400' ERP component than controls in response to viewing direct eye gaze from static images of female faces. This result suggests that the response to eye gaze in relatives of autistic probands was delayed and less persistent. Elasabbagh concluded that atypical response to direct gaze was a reliable feature of the infant BAP.

1.3.5.8 MEG Studies and the BAP

Other studies have examined neurofunctional correlates of the BAP using magnetoencephalography (MEG). MEG is a neuroimaging technique that provides information about brain activity by measuring magnetic fields generated by electrical

currents within neurones. Rojas et al. (2011) took MEG recordings of 21 parents of autistic probands and 21 adult controls reporting a reduction in gamma-band responses in the ASC parent group, similar to the responses of children diagnosed with autism (e.g. Wilson et al., 2007). Gamma bands are high frequency electromagnetic activity > 30 Hz that are sometimes detected during MEG recordings and are believed to play a role in a number of cognitive functions, such as selective attention and working memory, as well as is being associated with connectivity. In addition to reporting reduced gamma band activity in the ASC parent group, Rojas et al. also found that a measure of gamma band activity correlated with the 'communication' subscale of the AQ in ASC parents. Rojas et al. reported that the behavioural measures of the BAP (SRS and AQ) did not strongly distinguish groups whilst biological markers derived from the MEG recordings seemed to be more sensitive at picking up differences between autistic probands, first degree relatives of autistic probands and controls.

1.3.5.9 Structural MRI and the social brain in ASC relatives

A very small number of studies have investigated structural differences in the social brain of ASC relatives. A study by Dalton et al. (2007) found a significant reduction in the volume of the amygdala in siblings of people with ASC compared to controls. However, no group difference in amygdala volume was detected between ASC parents and controls in a study by Peterson et al. (2006). There is therefore currently limited evidence for structural differences in brain regions connected to the social brain in the relatives of autistic probands

1.3.5.10 Other structural neuroimaging studies of ASC relatives

Other structural MRI studies include Rojas et al. (2004) who reported that the parents of children with ASC had significantly larger left hippocampus volumes compared to controls from the general population. However, these results failed to replicate in a study by Peterson et al. (2006). Peterson and colleagues carried out a structural MRI study of gray matter in the parents of autistic probands. The scans revealed differences, relative to adult controls, in regions functionally associated with social-cognitive and motor processes that are impaired in ASC. Using voxel-based morphometry, Peterson et al. reported an increase in gray matter in the inferior and medial frontal gyri and cerebellum. Both Rojas et al. (2004) and Peterson et al. (2006) reported no significant differences in total brain volume between experimental and control groups. These studies, however, contrast with Palmen et al. (2005) who found no significant differences in the volume of *any* brain regions between ASC parents and controls using structural MRI. Finally a structural MRI study by Branchini et al. (2009) reported no significant difference in total/ regional corpus callosum area between the siblings of children with ASC and age/IQ-matched controls.

Structural investigations of the BAP also include Diffusion Tensor Imaging (DTI). A study by Barnea-Goraly et al. (2010) used Diffusion Tensor Imaging to investigate differences in white matter in children with ASC, their unaffected siblings and controls. Barnea-Goraly and colleagues carried out a whole brain analysis using tract-based spatial statistics and found significantly reduced white matter fractional anisotropy values in

both the ASC and ASC sibling group, relative to age and IQ-matched controls. Areas where aberrant white matter was detected included the medial prefrontal and superior temporal regions and the temporo-parietal junctions. Reductions were found in axial diffusivity but not radial diffusivity suggesting that the alterations were in fiber coherence rather than myelination. However, no significant correlations were found between white matter functional anisotropy/ axial diffusivity and ASC symptomatology. Furthermore, unaffected siblings were excluded if they displayed behavioural features of the BAP using the FHI. Therefore, DTI measures may be more sensitive to subtle differences in the first degree relatives of autistic probands and controls indicative of the BAP at a biological/ neurostructural level.

1.3.5.11 Summary

Neurofunctional and neuroanatomical studies of autistic probands and their relatives using neuroimaging techniques such as fMRI, sMRI, ERP, MEG and DTI have started to reveal important differences in brain structure, activity and connectivity in and between regions of the brain. Such studies have proven essential in furthering our understanding of the neural correlates of the perceptual and cognitive aspects of ASC (e.g. visual divided attention and social cognition; see Table 1.3). Future studies should continue to search for neural underpinnings of BAP expression at a cognitive and behavioural level. These studies are still in their infancy and more neuroimaging research is required to determine the extent to which autistic probands and their first degree relatives share atypicalities in brain structure and function. Furthermore, these studies warrant

replication in order to protect against possible publication biases in the neuroimaging research literature (see Ioannidis, 2011).

1.4 Summary of findings and future directions; looking ahead to chapters two to seven

1.4.1 Summary of findings

This chapter summarises research studies that have taken place over the last 20-30 years on the BAP from multiple, mutually reinforcing categories of analysis. The list of prospective traits for the BAP discussed here is not exhaustive and in the future must include a more thorough and diverse examination of domains of functioning associated with ASC such as sensory hypersensitivity and motion processing/ detection (e.g. see Bertone et al., 2003; Bonnel et al., 2003; Gepner and Féron, 2009; Gepner and Mestre, 2002 and Leekam et al., 2007). Nevertheless, a wide variety of traits has been examined for inclusion in the BAP; this firstly includes the possibility of an early emerging BAP in the younger infant siblings of children with ASC. Candidate traits include language delay and social deficits such as atypicalities in gaze shift patterns, reduced requesting behaviour, initiation of joint attention and responding to joint attention (see Table 1.1). Studies also report early problems in visually disengaging from stimuli, whilst more research is needed investigating executive function and ToM in at-risk infant siblings. However, many of the research studies conducted on at-risk siblings in this chapter have not reassessed this experimental group when the siblings are older than three years of age

so it is not clear whether autistic traits displayed in at-risk siblings are part of the full ASC phenotype or isolated traits indicative of the BAP. This methodological constraint does not apply for older siblings and parents of autistic probands.

In older siblings and parents, positive findings at a behavioural level have been most consistently reported for pragmatic language skills, social responsiveness and other areas of reciprocal social interaction. More research needs to examine restricted, repetitive interests in the relatives of people with ASC. Of particular interest is the question of whether the BAP is restricted to specific aspects of this behavioural domain, such as circumscribed interests or a rigid/ perfectionistic style, or whether it applies more broadly, including repetitive motor activities and resistance to change.

At a cognitive level, the BAP has most consistently been found for social cognition e.g. complex mental state recognition, emotion recognition and face processing strategy. It is less clear whether executive functioning is part of the BAP. Findings in this area have been less consistent and a number of studies finding impairments did not appropriately match experimental and control groups for IQ (e.g. Hughes et al., 1997; Piven and Palmer, 1997). In contrast, a number of studies investigating social cognition in ASC relatives matched control groups for IQ (e.g. Baron-Cohen and Hammer, 1997; Dorris et al., 2004; Gokcen et al., 2009), although there are exceptions (Losh and Piven, 2007). Results are also mixed for studies assessing local visual processing in the relatives of individuals with ASC. Other areas of cognition requiring further research include divided attention and engagement/ disengagement of attention to social and non-social stimuli. It

should be noted that the conflicting results reported in this chapter must be set in the wider context of ASC research, where deficits in cognitive domains such as executive function or ToM are neither specific nor universal in people clinically diagnosed with ASC. Lastly, interview and questionnaire-based measures indicate an elevated rate of personality traits in the BAP, including 'aloof', 'rigid' and 'hypersensitive' as well as elevated rates of other psychiatric conditions in ASC families, such as anxiety and depression.

1.4.2 Finding promising endophenotypes; Chapter Two

An endophenotype is a measurable and heritable characteristic associated with a condition that is more proximal to the genotype than the clinical phenotype (Gottesman and Gould, 2003). For this reason they have the potential to decrease phenotypic heterogeneity and increase the power to detect vulnerability genes for a complex psychiatric condition. Such an approach has been advocated by researchers in the field of autism genetics (e.g. Leboyer et al., 1998; Le Couteur et al., 1996; Smith et al., 2009; Weiss, 2009) as well as in behavioural genetics more broadly (e.g. deGeus, 2002; de Geus and Boomsma, 2001; Gottesman and Gould, 2003). The evidence as collated in Table 1.1 and 1.2 provides pointers to the most promising behavioural and cognitive endophenotypes for autism (including pragmatic difficulties, language delay, reduced social responsiveness, poorer social skills, ToM difficulties, emotion recognition difficulties and poorer performance on visual divided attention/ social orienting tasks). Table 1.3 also gives preliminary suggestions for endophenotypes at the neural level.

Molecular genetic studies of ASC have currently been most successful in detecting rare gene variants and rare copy number variations (CNV[9]) with large effects (Abrahams and Geschwind, 2008; Freitag et al., 2010; Pinto et al., 2010). Studies examining the role of common gene variants affecting the risk for ASC have been less consistent and are hampered by lack of replication (e.g. Anney et al., 2010). Common ASC gene variants are likely to be of weak effect, and typically require very large sample sizes in order to have sufficient power to be detected. If studies on the BAP detect similar but milder manifestations of autistic traits in the relatives of autistic probands, this opens up the possibility to include relatives with sub-threshold autistic traits in genetic linkage and association studies that explore common inherited variants linked to ASC. It is therefore extremely important to obtain reliable, quantitative measures of autistic traits and related phenotypes that are likely to be under genetic influence, so that these measures can be applied in future genetic studies of ASC. Some previous studies using quantitative measures of autistic traits have reported significant association or linkage findings using both general population (e.g. Pourcain et al., 2010) or clinical samples (e.g. Duvall et al., 2007), illustrating the usefulness of this approach.

In chapter two the BAP will be explored in the parents of children with ASC by using a quantitative self-report measure of empathy and a performance-based measure related to empathy that assesses basic facial emotion recognition. The review of BAP research reported in this chapter has implicated a number of empathy-related measures in the BAP and so the study reported in the next chapter empirically examines empathy and emotion

[9] CNV are large fragments of DNA greater than 50 kilobases long that get inserted into or deleted from chromosomes (Abrahams and Geschwind, 2008).

recognition in ASC parents versus adult controls, as well as in adults with ASC. Chapter two is the first empirical study of this thesis to investigate quantitative measures associated with ASC symptomatology in ASC parents, as part of the BAP. These studies are instrumental in determining which aspects of the BAP show the most promise for inclusion in genetic studies. Chapter two is also the first empirical study of the thesis to explore two possible endophenotypes for ASC: self-rated empathy and emotion perception.

1.4.3 Differentiating between simplex and multiplex autism families; Chapters Three to Six

Future research into the BAP could also help to better understand the genetic mechanisms underpinning ASC and the BAP. The results of a number of recent autism genetic studies indicate that there is an important distinction between single-incidence (simplex) autism families and multiple-incidence (multiplex) families. Firstly, the aetiology of simplex autism may be more strongly influenced by rare, de novo genetic mutations or CNV of large effect compared to multiplex autism (Levy et al., 2011; Marshall et al., 2008; O'Roak et al., 2012; Sanders et al., 2012; Sebat et al., 2007). For example, using comparative genomic hybridization, Sebat et al. (2007) identified a number of de novo CNV that were significantly associated with ASC and found in 10% of probands with simplex autism, 3% of probands with multiplex autism and 1% of controls. Conversely it is hypothesised that the aetiology of multiplex autism is more strongly influenced by multiple common, inherited gene variants of weak effect shared by other members of the

family, although the current evidence for the role of these variants in ASC aetiology is sparse and results have not yet been replicated (Anney et al., 2010; Wang et al., 2009). Thus, the clear evidence for the existence of a BAP in the research literature provided in the beginning of this chapter needs to be reconciled with these autism genetic findings. De novo genetic events in the proband that are not inherited from either parent can not be expected to contribute to the BAP in other relatives. If different types of genetic variation are making different contributions to simplex and multiplex autism, with de novo genetic events playing a major role in simplex autism then one could hypothesise that the expression of BAP should be different in multiplex versus simplex autism families with the BAP being largely restricted to the unaffected relatives from multiplex families. A number of recent studies have suggested that this is the case; sub-threshold autistic traits aggregate in multiplex autism families and occur less frequently in simplex autism families (e.g. Constantino et al., 2006, 2010; Virkud et al., 2009) and it is thought that these findings reflect differential modes of genetic transmission of autistic traits in simplex and multiplex families. However, these studies have largely used a single measure of autistic traits only and thus only one measurement type (self or informant-report scale, such as the SRS). These studies therefore do not provide a very full picture of the BAP. More studies are needed examining the expression of the BAP in simplex and multiplex families in order to further test the hypothesis of differential modes of genetic transmission in these families.

In chapters three to six the BAP is explored in the parents of multiplex versus simplex autism families and controls. Firstly, these studies aim to reconcile some of the

inconsistent findings in the research literature on the BAP by stratifying the ASC relative group according to their affiliation to simplex or multiplex autism families. Secondly, these studies aim to examine whether the expression of autistic traits and related phenotypes in multiplex and simplex parents is consistent with the hypothesis that differential genetic mechanisms operate in simplex and multiplex autism families, as just described. These studies also use a wider range of measures than previous studies of the BAP in multiplex versus simplex autism (e.g. Bölte and Poustka, 2003, Constantino et al., 2006, 2010; De la Marche et al., 2011, Virkud et al., 2009). In chapter three these measures will be described in detail along with the general methods for these studies into the BAP in multiplex versus simplex autism, reported in chapters four to six. The methods include participant recruitment and eligibility, the testing procedure, the proband diagnosis verification criteria, the simplex/ multiplex classification criteria and the sample characteristics. In chapter four the BAP will be explored by comparing simplex parents, multiplex parents and controls on self-report measures of autistic traits and related phenotypes, namely empathy and systemising (Baron-Cohen, 2002, Baron-Cohen et al. 2003, Baron-Cohen and Wheelwright 2004). Examination of the BAP in multiplex versus simplex autism parents continue in chapter five using performance-based tasks of empathy and systemising. Finally, in chapter six parental psychopathology and family psychiatric history is explored in multiplex versus simplex autism families using two measures: a self-report questionnaire that assesses a wide range of psychiatric problems and a short parental interview about mental health problems in the family. In summary, the studies reported in chapters four to six aim to provide the most comprehensive overview of the BAP in multiplex and simplex autism relatives to date.

1.4.4 Furthering our understanding of the association between social and non-social domains of ASC; Chapter Seven

BAP research can make an important contribution to understanding the relationships between the social and non-social behavioural and cognitive domains characterising ASC. This can be achieved by examining whether the expression of the BAP occurs as a whole, or only to particular aspects of the BAP. This should have implications for understanding whether the social and non-social aspects of ASC have distinct causes. If the social or non-social features of ASC appear in isolation amongst the relatives of autistic probands, then this implies that they may be fractionable (i.e. have distinct causes; Happé and Ronald, 2008). Chapter seven will scrutinise whether this may be the case for the first time in the parents of children with ASC by examining the relationships between social and non-social autistic traits and related phenotype in these relatives.

To better understand these associations it is important to consider relationships across different levels of analysis, such as between: (1) observational reports of behaviour, and (2) performance-based measures and instruments that systematically examine cognition. Chapter seven also explores relationships between different levels of analysis in ASC parents, by examining the links between self-report scales of autistic traits and related phenotypes and cognitive performance-based measures associated with empathy and systemising.

1.4.5 Thesis conclusions; chapter eight

This thesis concludes in chapter eight with a summary of findings across chapters one to seven, as well as describing how this thesis advances previous research into the BAP. Furthermore this chapter takes a closer look at the limitations of conducting studies on the BAP, before proceeding to discuss the theoretical implications of the research described in this thesis; this includes scrutinising the validity of some of the phenotypes that emerge as potential candidates for ASC endophenotypes from the empirical chapters described in chapters two to six. This chapter ends by suggesting future avenues of research on the BAP and discussing some of the possible practical implications of this research.

1.5 Aims, Predictions and Hypotheses

The hypotheses and aims for the following empirical chapters are summarised below:

1.51 Chapter Two

This study aims to explore measures of empathy in the first-degree genetic relatives of autistic probands for the first time by considering whether empathy and emotion recognition are part of the BAP. This study also aimed to replicate previous findings of empathy and emotion recognition difficulties in adults with ASC. It is predicted that self-rated empathy and emotion recognition difficulties will be found in adults with ASC and,

to a milder degree, in the unaffected parents of children with ASC. This would be indicated by significant differences between adults with ASC/ parents of children with ASC and controls on the empathy measures used. These significant differences are hypothesised to be caused by a shared genetic vulnerability to ASC in family members, which in unaffected parents manifests itself as the BAP.

Finally, this study aimed to test if there are sex differences in each of the three groups (adult controls, parents of children with ASC and adults with ASC) on self-report and performance-based measures of empathy. Based on previous research, it is predicted that females outperform males on measures of empathy in the general population, which is hypothesised to be caused by sex differences in both biological and sociocultural factors (Baron-Cohen, 2003). There are no previous studies that have examined sex differences in empathy in adults with ASC and parents of children with ASC, so no predictions and hypotheses are made for these samples.

1.5.2 Chapters Three to Six

These studies aimed to reconcile some of the inconsistent findings in the BAP research literature by stratifying the parents of autistic probands according to their affiliation to simplex or multiplex autism families. These studies also aim to examine whether there are differences in the aggregation of autistic traits and related phenotypes in the unaffected parents of multiplex autism families versus simplex autism families and, where possible, controls. In chapter four, it is predicted that significantly higher self-rated

autistic traits and related phenotypes (low empathy/ high systemizing) will be found in multiplex parents versus simplex parents and controls. In chapter five, it is predicted that significantly poorer performance on social cognition tasks and significantly superior performance on a perceptual attention to detail task will be found in multiplex parents versus simplex parents. In chapter six, it is predicted that psychiatric problems will aggregate in multiplex family members compared to simplex family members, particularly traits consistent with affective disorders, avoidant personality disorder and ADHD. These predictions are united by the behaviour genetic hypothesis, stated previously, that differential genetic mechanisms have differential causal effects on the autism phenotype in multiplex and simplex autism, with inherited genetic variants conferring higher risk in multiplex autism and de novo genetic variants conferring higher risk in simplex autism.

1.5.3 Chapter Seven

This study aimed to assess the phenotypic relationships between the social and non-social symptom domains of ASC in the unaffected parents of autistic probands; firstly across the entire sample of ASC parents and secondly by comparing parents with and without high autistic traits in the social and non-social domain. Based on previous research, it is predicted that characteristics associated with the social symptom domain will aggregate separately from the characteristics associated with the non-social symptom domain, especially in parents with high autistic traits. Separate aggregation of social and non-social characteristics in ASC parents are hypothesised to be caused by independent and

distinct causes acting on the social and non-social symptom domains of ASC that appear in isolation in first-degree relatives of autistic probands who are at a greater genetic vulnerability to ASC than the general population.

Chapter Two

Empathy and emotion recognition in people with ASC, first-degree relatives and controls.[10]

[10] This chapter is adapted from: Sucksmith, E., Allison, C., Baron-Cohen, S., Chakrabarti, B. & Hoekstra, R.A. (2013). Empathy and emotion recognition in people with autism, first-degree relatives and controls. *Neuropsychologia,* 51(1), 98-105.

2.1 Abstract

Empathy is the lens through which we view others' emotion expressions, and respond to them. In this chapter, empathy and facial emotion recognition were investigated in adults with autism spectrum conditions (ASC; N=314), parents of a child with ASC (N=297) and IQ-matched controls (N=184). Participants completed a self-report measure of empathy (the Empathy Quotient [EQ]) and a modified version of the Karolinska Directed Emotional Faces Task (KDEF) using an online test interface. Results showed that mean scores on the EQ were significantly lower in fathers ($p < 0.05$) but not mothers ($p > 0.05$) of children with ASC compared to controls, whilst both males and females with ASC obtained significantly lower EQ scores ($p < 0.001$) than controls. On the KDEF, statistical analyses revealed poorer overall performance by adults with ASC ($p < 0.001$) compared to the control group. When the 6 distinct basic emotions were analysed separately, the ASC group showed impaired performance across five out of six expressions (happy, sad, angry, afraid and disgusted). Parents of a child with ASC were not significantly worse than controls at recognising any of the basic emotions, after controlling for age and non-verbal IQ (all $p > 0.05$). Finally, results indicated significant differences between males and females with ASC for emotion recognition performance ($p < 0.05$) but not for self-reported empathy ($p > 0.05$). These findings suggest that self-reported empathy deficits in fathers of autistic probands are part of the broader autism phenotype. This study also reports new findings of sex differences amongst people with ASC in emotion recognition, as well as replicating previous work demonstrating empathy

difficulties in adults with ASC. The use of empathy measures as quantitative endophenotypes for ASC is discussed.

2.2 Introduction

As already described, ASC are neurodevelopmental in origin, and are characterized by difficulties with social interaction and communication, together with unusually restricted, repetitive behaviours and interests (APA, 2000; WHO, 1993). ASC involve a large number of behavioural manifestations that vary considerably across individuals and development. It is therefore important to test neurocognitive models that reduce these behavioural symptoms to a small number of underlying processes.

One of the earliest and most influential neurocognitive models for ASC is the theory of mind (ToM)/'mind-blindness' hypothesis. This states that the behaviour observed in ASC is due to difficulties representing the contents of one's own and other people's minds (Baron-Cohen, 1995). Successful social interaction requires the ability to attribute mental states to others in order to explain and predict their behaviour. Early studies assessing ToM in ASC and typically developing children primarily focused on the application and understanding of beliefs (Baron-Cohen et al., 1985; Perner et al., 1989), intentions (Phillips et al., 1998) and pretence (Baron-Cohen, 1987; Leslie, 1987; Scott and Baron-Cohen, 1996). The ToM hypothesis can explain the social features of ASC but never set out to explain its non-social features. The hypothesis can also only explain the earliest symptoms of ASC by reference to simpler precursors of ToM, such as joint-attention and pretence (Pellicano, 2011). More recently, empathy has been proposed as a broader neurocognitive construct underlying the social and communicative difficulties observed in people with ASC (Baron-Cohen, 2002). Empathy extends the ToM hypothesis by not

only focusing on the attribution of another person's mental state but also on the capacity to respond to another's mental states with an appropriate emotion (Baron-Cohen, 2002). It therefore includes both a cognitive component (identifying other people's beliefs, desires, intentions etc.) and an affective component (responding to other people's mental states with an appropriate emotion) (Baron-Cohen and Wheelwright, 2004; Chakrabarti and Baron-Cohen, 2006a).

The present study explores the hypothesis that the social communicative features of ASC entail empathy difficulties. This is tested using a self-report measure of empathy, the Empathy Quotient [EQ] (Baron-Cohen and Wheelwright, 2004). Self-report scales are useful in adulthood but one of their limitations is that a participant's responses may not accurately reflect their true capabilities. Therefore, this study also includes a test of facial emotion recognition, as a performance measure.

Previous studies of the ability to recognize facial expressions of emotion in ASC have produced inconsistent results. Many studies have identified deficits in specific, negatively valenced expressions, including fear (Howard et al., 2000; Pelphrey et al., 2002), anger (Giola and Brosgole, 1988) and disgust (Golan et al., 2006) whilst other studies have identified impairments across all negative basic emotions (Ashwin et al., 2006). Other studies have not found differences in basic emotion recognition performance in ASC (Adolphs et al., 2001; Loveland et al., 2008; Rutherford and Towns, 2008). A review by Harms et al. (2010) concluded that these discrepant findings were largely attributable to differences in IQ, task demands (static versus dynamic facial stimuli) and the types of

dependent variables measured (electrophysiological/ behavioural). Other studies have attributed the discrepant findings to variability in the intensity of emotions used as task stimuli (Law Smith et al., 2010).

As reviewed in chapter one, a proportion of 'unaffected' relatives of people with ASC exhibit milder features of the full autism phenotype (the BAP; Bolton et al., 1994). These characteristics occur at behavioural, cognitive and neurophysiological levels. However, only a small number of features have consistently been found to occur frequently in the unaffected relatives of ASC probands. These include social communication difficulties and reduced performance on measures of social cognition (Wheelwright et al., 2010; Sucksmith et al., 2011 or refer to chapter one). Previous studies of the BAP have included emotion recognition performance. Some of these have found first-degree relatives to exhibit milder difficulties in recognizing facial expressions (Losh et al., 2009; Palermo et al., 2006; Wallace et al., 2010; but see Bölte and Poustka, 2003). To date, there have been no studies assessing whether the relatives of individuals with ASC self-report less empathy compared to a control group.

The primary aim of this study was to assess whether parents of children with ASC show reduced self-reported empathy, as well as emotion recognition difficulties, compared to IQ-matched controls, as part of the BAP. Secondly, we sought to replicate previous findings of difficulties with empathy and emotion recognition in adults with ASC. Finally, we tested if there are sex differences in each of the three groups (adult controls, parents of children with ASC, and in adults with ASC) on self-report and performance

measures of empathy. Previous studies suggest significant sex differences in the general population for empathy measures, with females on average reporting higher empathy and outperforming males on performance-based tasks of empathy (Baron-Cohen and Hammer, 1997; Baron-Cohen and Wheelwright, 2004). Likewise, a small number of studies suggest sex differences within ASC itself on various behavioural measures (Bölte et al., 2011; Lai et al., 2011), but this remains an under-researched area, largely due to difficulties in recruiting enough female participants with ASC. In our online study it was possible to recruit a relatively large sample of both males and females with a clinical ASC diagnosis.

2.3 Methods

2.3.1 Participants

Parents of children with an ASC diagnosis and adults with an ASC diagnosis were recruited from the Cambridge University Autism Research Centre volunteer database (www.autismresearchcentre.com). Recruitment of participants to this database has ethics approval from the Cambridge University Psychology Research Ethics Committee. During the registration process parents confirmed via self-report if they have a diagnosis of ASC, and we excluded those who did. They also had to report at least one child with a diagnosis of ASC from a clinician based on DSM-IV or ICD-10 criteria. Adults with ASC self-reported that they had been diagnosed by an experienced clinician according to DSM-IV or ICD-10 criteria. Control participants were also recruited online, via a

different portal (www.cambridgepsychology.com). During the registration process, control participants self-reported that they do not have an ASC diagnosis and that they were not the parent of a child with an ASC diagnosis. We excluded control participants with any other psychiatric diagnosis.

In total, 187 adult controls (93 males, 94 females), 310 parents of children with ASC (38 males, 272 females) and 329 adults with ASC (161 males, 168 females) completed the EQ. These groups did not significantly differ on non-verbal IQ ($p = 0.34$) measured using an online adaptation of the Raven's Progressive Matrices (RPM; Raven et al., 1996). After data cleaning and careful matching for non-verbal IQ ($p = 0.19$), the following samples sizes were available for the KDEF test: 184 adult controls (92 males, 92 females) 297 parents (36 males, 261 females), and 314 adults with ASC (164 males, 150 females).

Approximately equal numbers of males and females were recruited in the control and ASC groups for both measures. In the parent group, there were a higher number of mothers than fathers on both measures, probably reflecting previous findings of higher response rates in females compared to males (Gosling et al., 2004). The mean age of participants completing each measure differed slightly across groups; the parents of children with ASC were older than both controls and adults with ASC. Nevertheless, the range of ages in the ASC parent group was similar to controls and adults with ASC (ASC parents: 24-61 years, ASC: 16-70 and Controls: 19-65). Table 2.1 displays descriptive data for the three groups of participants that completed the EQ and KDEF, including sample sizes, mean ages and IQ scores.

Table 2.1: *Descriptive data for group analysis of the EQ and KDEF*[11].

	EQ			KDEF		
	N	**Mean age (SD)**	**Mean non-verbal IQ (SD)**	**N**	**Mean age (SD)**	**Mean non-verbal IQ (SD)**
Control	187	34.3 (10.76)	52.7 (3.58)	184	34.4 (10.84)	52.7 (3.64)
ASC Parent	310	41.0 (6.34)	52.1 (3.56)	297	41.0 (6.43)	52.1 (3.46)
ASC	329	35.5 (11.03)	52.3 (4.24)	314	35.7 (11.25)	52.5 (4.11)

2.3.2 Materials and procedure

After registering online and consenting to take part in research, participants were asked to complete the different measures in their order of preference. These included the Empathy Quotient (EQ; Baron-Cohen and Wheelwright, 2004) which consists of 40 items, where participants respond to each item using a 4 point Likert scale ('strongly agree', 'slightly agree', 'slightly disagree' and 'strongly disagree'). An empathic response to an item is given a score of '1' or '2' depending on the strength of the response. 21 out of the 40 scored items are reversed to avoid response biases. Other responses are given a score of '0'. Scores on each item are summed providing a total score between 0 and 80. There were no missing values.

The EQ has excellent test-retest reliability ($r = 0.97$, $p < 0.001$; Baron-Cohen and Wheelwright, 2004) and good construct validity, correlating positively with a performance-based measure of social cognition (the 'Eyes' task; $r = 0.294$, $p < 0.05$;

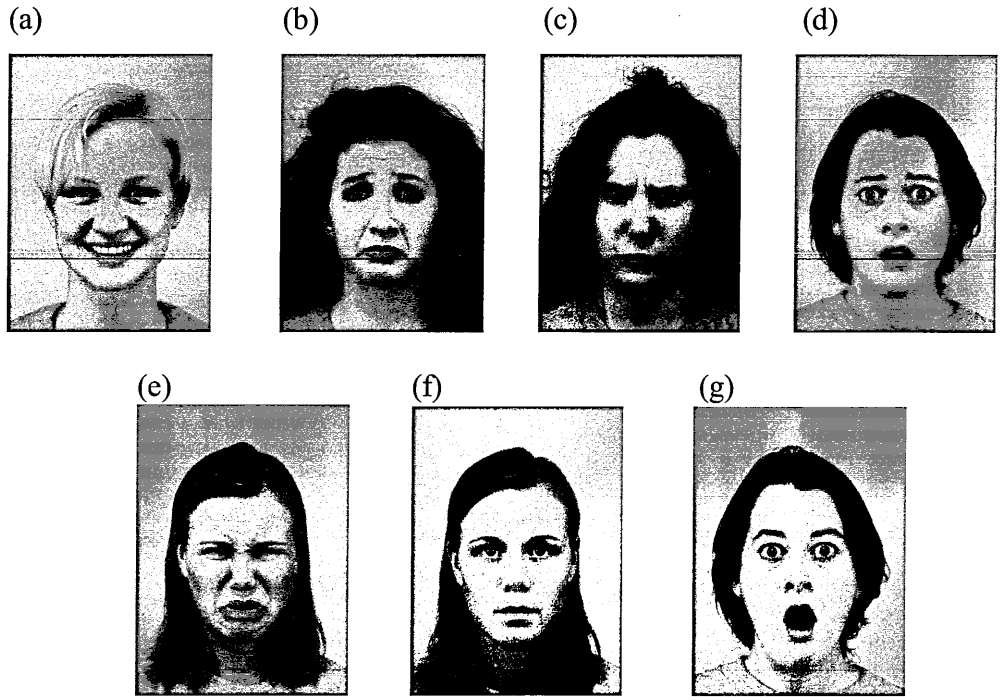
[11] EQ; Empathy Quotient, KDEF; Karolinska Directed Emotional Faces Task.

Lawrence et al., 2004). It also has high internal consistency (Cronbach's alpha = 0.92; Baron-Cohen and Wheelwright, 2004). Currently the most comprehensive assessment of the dimensionality of the EQ using a Rasch and Confirmatory Factor Analysis suggests that the EQ is a unidimensional measure (Allison et al., 2011).

Participants also completed a modified version of the Karolinska Directed Emotional Faces Task (KDEF; Lundqvist et al., 1998) using the online test interface. Participants were shown 140 photographs of people's faces expressing one of six 'basic' emotions (happy, sad, angry, afraid, disgusted and surprised) as well as a neutral expression (see Figure 2.1). There were 20 photographs in total for each expression. For each photograph, participants were asked to select which of the seven words described the emotion being expressed. Participants were told they had 20 seconds to respond to each photograph and they must answer as quickly and accurately as possible. Results provide an accuracy score and response time (for correct trials only) for each facial expression of emotion. The stimuli used in the KDEF have been validated on emotional content, intensity and arousal and have good test-retest reliability (Goeleven et al., 2008). Furthermore, the KDEF stimuli set have good ecological validity, unlike schematic or computerized faces.

All data were rigorously checked prior to the data analyses. 22 data points were identified as outliers (> 3 standard deviations from the group mean) and so were removed from the data set, resulting in the final sample size of 314 adults with ASC, 297 parents and 184 control participants.

Figure 2.1: *Examples of Stimuli used in the KDEF*[12] *(Lundqvist et al., 1998);(a) happy; (b) Sad; (c) Angry; (d) Afraid; (e) Disgust; (f) Neutral; (g) Surprise.*



Finally, participants used the online test interface to complete an online adaptation of the RPM, a measure of non-verbal intelligence (Raven et al., 1996). The RPM consists of 60 items displaying geometric designs of varying complexity that contain a missing piece. Participants had to choose from a selection of designs to complete the pattern. Performance on the online RPM was used so that groups could be matched on non-verbal IQ; this ensures that the relationship between group status and the empathy/emotion recognition measures is undistorted by non-verbal IQ and that any significant differences found reflect selective difficulties in behaviour/cognition. RPM accuracy score was also

[12] KDEF; Karolinska Directed Emotional Faces Task.

used as a covariate in data analyses to remove any covariance from the outcome measures that could be attributed to variation in non-verbal cognitive ability.

2.3.3 Statistical Analyses

Adults with ASC, parents of children with ASC and the control group were compared on mean EQ scores using a univariate analysis of covariance (ANCOVA) with non-verbal IQ and age used as covariates. Previous studies have reported sex-specific expression of the BAP (Happé et al., 2001; Constantino et al., 2006) and sex differences on measures of empathy (Baron-Cohen and Wheelwright, 2004), so sex was also used as a between-subjects factor in the data analyses.

For the KDEF, two dependent variables were analysed. First, accuracy was used, in line with previous research on facial emotion recognition in ASC (Ashwin et al., 2006; Bölte and Poustka, 2003). Secondly, 'accuracy-adjusted response time' (ART) was used which is likely to be a more sensitive measure as it controls for a potential speed-accuracy trade-off (see Mevorach et al., 2006 and Sutherland and Crewther, 2010 for similar approaches). Accuracy scores showed high ceiling effects, with distributions significantly deviating from the normal distribution. Therefore, non-parametric Kruskal-Wallis tests were carried out on accuracy scores for each emotion, with group used as the fixed factor. For emotions that showed significant differences, planned follow-up Mann-Whitney U tests were carried out between ASC parents and controls and between ASC adults and controls.

Accuracy-adjusted response times were calculated for each emotion by dividing the mean response time for correct items by the fraction of items answered correctly. This ratio provides a degree of adjustment for potential speed-accuracy tradeoffs. Adults with ASC, parents of children with ASC and the control group were compared on this dependent variable using a mixed analysis of covariance (ANCOVA). This test was used to compare groups on overall mean accuracy-adjusted response time across all emotions. Follow up ANCOVAs with planned contrasts were then carried out to compare groups on each emotion separately. In these analyses, sex was again included as a fixed factor and non-verbal IQ and age used as covariates.

2.4 Results

Table 2.2: *Descriptive data for group analysis of the EQ and performance on the KDEF, separated by gender*[13].

	Males			Females		
	Control	**ASC parent**	**ASC**	**Control**	**ASC parent**	**ASC**
EQ						
N	93	38	161	94	272	168
Mean Score (SD)	37.7 (13.5)	32.2 (13.5)	17.5 (10.5)	48.5 (14.1)	46.6 (17.7)	18.2 (8.9)
KDEF						
N	92	36	164	92	261	150
Mean accuracy per emotion (/20) (SD)	17.49 (1.18)	17.34 (1.38)	16.60 (1.80)	17.80 (1.21)	17.71 (1.03)	16.70 (1.76)
Mean ART (ms) per emotion (SD)	2885.44 (745.14)	3113.44 (794.68)	3577.71 (1091.95)	2637.13 (621.80)	2774.75 (708.09)	3168.45 (1071.96)

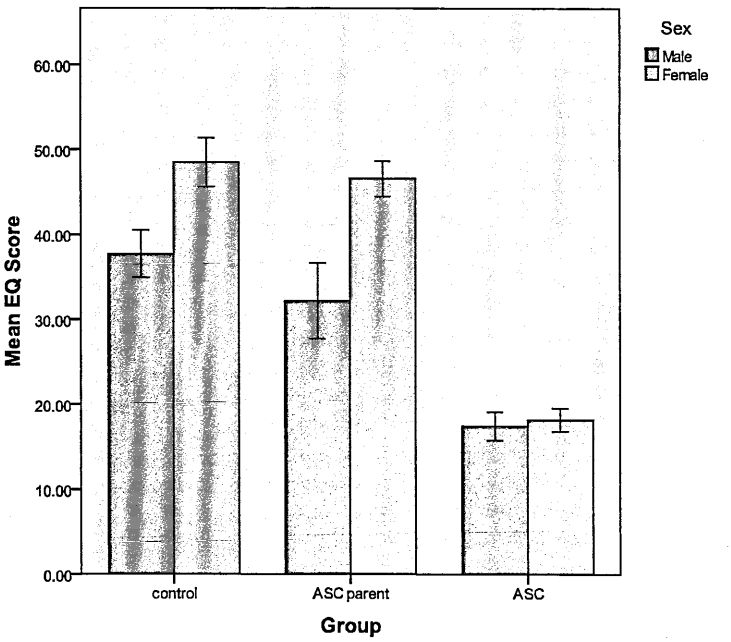
[13] EQ; Empathy Quotient, KDEF; Karolinska Directed Emotional Faces Task, ASC; Autism Spectrum Conditions, ART; Accuracy-adjusted Response Time.

2.4.1 Self-rated Empathy

Table 2.2 shows the mean EQ scores, standard deviations and available sample sizes for each group, separated by gender. A group × sex ANCOVA with age and non-verbal IQ as the covariates showed that age did not have a significant effect on mean EQ score ($F(1, 818) = 0.25$, $p > 0.05$), whilst non-verbal IQ was significantly related to mean EQ score ($F(1,818) = 10.59$, $p < 0.01$; Pearson's correlation coefficient $r = 0.11$, indicating a small effect size and thus a modest positive association between empathy and non-verbal IQ). Results also revealed a significant main effect of group ($F(2, 818) = 242.60$, $p < 0.001$). Contrast analyses suggested that the mean EQ score was significantly lower in adults with ASC ($p < 0.001$, $r = 0.51$) compared to the control group. The ANCOVA also revealed a significant main effect of sex ($F(1, 818) = 57.06$, $p < 0.001$, $r = 0.30$), with females obtaining higher scores than males. A significant interaction effect between group and sex on mean EQ score ($F(2, 818) = 14.64$, $p < 0.001$) was seen, suggesting that group effects are different for males and females (see Figure 2.2). Results from subsequent sex-specific ANCOVAs confirmed that both males and females with ASC reported significantly lower EQ scores on average than controls ($p < 0.001$. See Table 2.2 for mean scores). However, contrasts confirmed that fathers, but not mothers, of children with ASC reported a significantly lower mean EQ score compared to sex-specific controls (fathers: $p < 0.05$, $r = 0.32$; mothers: $p = 0.21$). Results from group-specific ANCOVAs confirmed that there was a non-significant difference between male and female EQ scores in adults with ASC ($p = 0.40$) but significant differences between males and females in the control group ($p < 0.001$, $r = 0.37$) and the ASC parent group (p

< 0.001, r = 0.07). This suggests that the significant group × sex interaction is partially caused by sex differences in mean EQ score amongst controls and ASC parents, whereas sex differences are absent in individuals with ASC (see Figure 2.2).

Figure 2.2: *Main effects of group and sex on mean EQ score*[14].



2.4.2 Emotion Recognition

2.4.2.1 Accuracy

Table 2.2 displays the descriptive data for performance on the KDEF task, which includes accuracy and accuracy-adjusted response time. Kruskal-Wallis tests were carried

[14] EQ; Empathy Quotient. Error bars depict the 95% confidence intervals.

out on accuracy scores for each emotion separately. These revealed a significant effect of group on four out of six basic emotions (happy, angry, afraid and disgust; $p < 0.001$) as well as the neutral expression ($p < 0.05$). Follow up Mann-Whitney U tests indicated that, compared to controls, adults with ASC were significantly less accurate at identifying these emotions (happy; $p < 0.05$, angry; afraid; disgust; $p < .001$) and at identifying neutral expressions ($p < 0.05$). Conversely, no significant differences were found between ASC parents and controls on these expressions (all $p > 0.05$).

2.4.2.2 Accuracy-adjusted response time

Accuracy-adjusted response times were logarithmically transformed to enable the use of parametric tests of statistical inference. After transformation the distribution was approximately normal in all groups; distributions of transformed accuracy-adjusted response times showed limited skew (Control; $S = 0.54$, ASC Parent; $S = 0.81$, ASC Adult; $S = 0.50$). A mixed analysis of covariance (ANCOVA) was carried out on mean accuracy-adjusted response times for each emotion, with group and sex as fixed factors and non-verbal IQ and age as the covariates. This revealed a significant main effect of group ($F(2, 787) = 40.83$, $p < 0.001$) and of sex ($F(1, 787) = 17.43$, $p < 0.001$, $r = 0.15$). The group × sex interaction effect failed to reach significance ($p > 0.05$), whilst the covariates (non-verbal IQ and age) had significant effects on accuracy-adjusted response time (non-verbal IQ; $F(1,787) = 9.54$, $p < 0.01$, age; $F(1, 787) = 16.43$, $p < 0.001$). Contrast analyses indicated that adults with ASC, but not ASC parents, had a significantly higher overall mean accuracy-adjusted response time compared to controls

(ASC adults; $p < 0.001$, ASC parents; $p > 0.05$). Contrasts also indicated significant differences in overall mean accuracy-adjusted response time between males and females across the three groups. Results from group-specific ANCOVAs indicated that the sex differences in accuracy-adjusted response time were significant in the control group ($p < 0.01$, $r = 0.19$), ASC parent group ($p < 0.05$, $r = 0.14$) and ASC group ($p < 0.001$, $r = 0.21$), with females outperforming males across all groups (see Figure 2.3).

Figure 2.3: *Main effects of group and sex on overall accuracy-adjusted response times on the KDEF*[15].

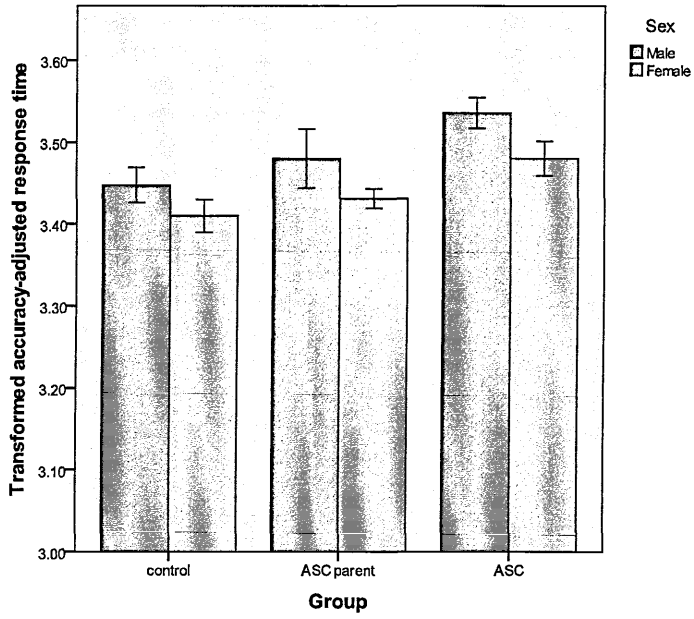


Figure 2.4 displays the main effect of group on accuracy-adjusted response times for individual facial expressions of emotion. Follow up ANCOVAs were carried out on mean

[15] KDEF; Karolinska Directed Emotional Faces Task. Mean accuracy-adjusted response times displayed are across all facial expressions of emotion. Error bars depict 95% confidence intervals.

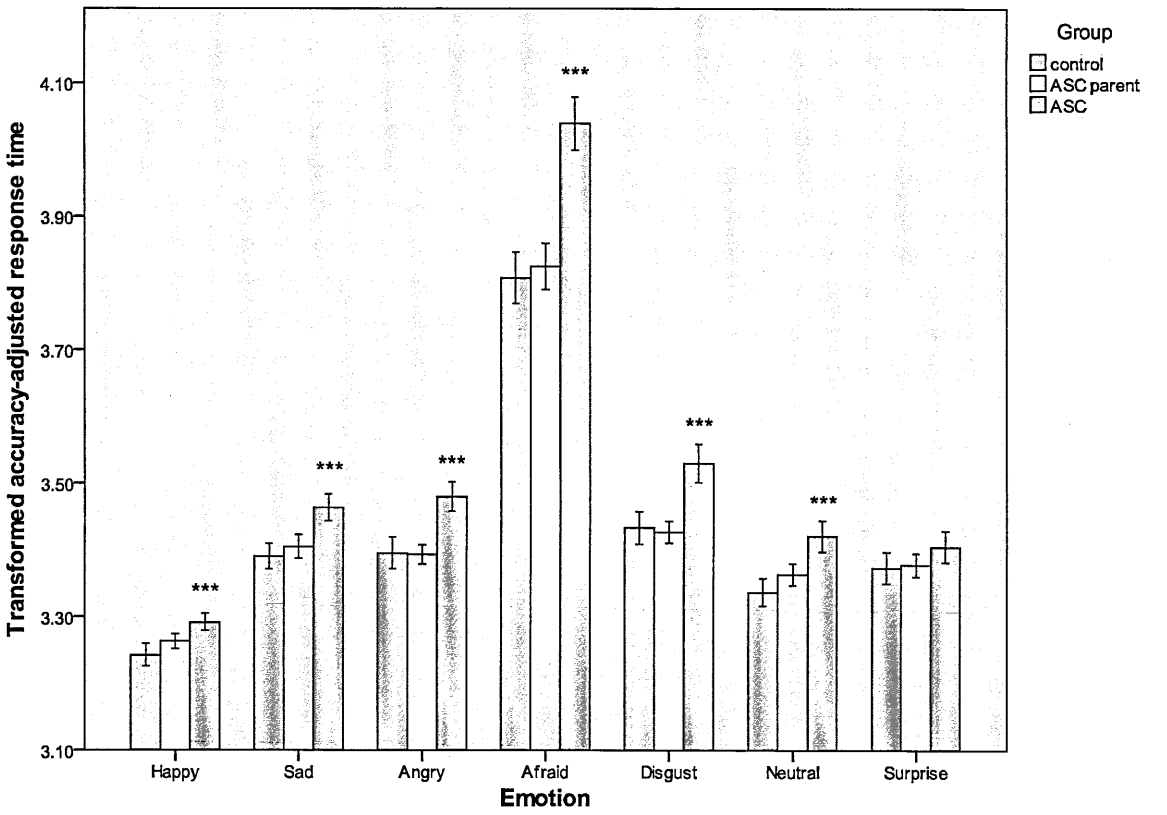
accuracy-adjusted response times for each emotion and the neutral expression, with group and sex as fixed factors and non-verbal IQ and age as the covariates. These analyses revealed a significant main effect of group on accuracy-adjusted response time for five emotions and the neutral expression (happy; sad; angry; afraid; disgust; neutral; $p < 0.001$). There was also a significant main effect of sex on accuracy-adjusted response time for five emotions (disgust; surprise; $p < 0.001$, sad; angry; $p < 0.01$, happy; $p < 0.05$). The non-verbal IQ covariate had a significant effect on the accuracy-adjusted response time for 3 facial expressions (afraid; $p < 0.001$, angry; disgust; $p < 0.05$), whilst the age covariate had a significant effect on the accuracy-adjusted response time for 4 facial expressions (happy; sad; neutral; $p < 0.001$, surprise; $p < 0.01$). There were no significant group × sex interactions (all $p > 0.05$). Contrast analyses indicated that the accuracy-adjusted response times of adults with ASC were significantly higher than the control group on 5 emotions and the neutral expression (happy; sad; angry; afraid; disgust; neutral; $p < 0.001$). These contrasts also indicated that there were no significant differences between parents of children with ASC and controls on accuracy-adjusted response times for each facial expression (all $p > 0.05$).

2.4.2.3 Correlations with EQ score

Lastly, the correlation between self-reported empathy and emotion recognition was explored in all three groups. Mean EQ scores and mean KDEF accuracy-adjusted response times were negatively correlated (ASC: $r = -0.16$, $p < 0.01$, ASC parents: $r = -0.15$, $p < 0.01$ and Controls: $r = -0.15$, $p < 0.05$). These significant correlations suggest

that the EQ and KDEF measure modestly overlapping constructs, such that people with relatively low self-rated empathy score somewhat lower on the performance test for emotion recognition.

Figure 2.4: *Main effect of group on mean accuracy-adjusted response times for separate facial expressions of emotion on the KDEF*[16].



[16] KDEF; Karolinska Directed Emotional Faces Task. Significant differences between control and experimental groups denoted by the asterisks: *** $p < 0.001$. Error bars depict the 95% confidence intervals.

2.5 Discussion

This study investigated empathy and facial emotion recognition in adults with ASC and in first-degree relatives (parents) of children with ASC. The evidence supports a BAP for self-rated empathy in fathers of children with ASC, but not for basic facial emotion recognition in parents of children with ASC. We also replicated previous studies reporting empathy and emotion recognition difficulties in adults with ASC, and found evidence for a difference between males and females with ASC on emotion perception. Each of these findings is discussed below.

Fathers but not mothers of children with ASC self-reported lower empathy than controls on the EQ. This suggests that lower self-reported empathy may be a reliable feature of the BAP in fathers only. Further research is needed to assess whether this sex-specific finding generalizes to other relatives, e.g. to brothers but not sisters of individuals with ASC. Some previous studies have suggested that certain aspects of the BAP may be especially prevalent in male relatives (Constantino et al., 2006). This study is the first to explore self-reported empathy in parents of a child with ASC. Equally, further research is needed to test if the absence of a self-reported empathy deficit in mothers is because they are over-estimating their true empathy level.

When analysing facial emotion recognition using a sensitive measure of performance (accuracy-adjusted response time), parents of children with ASC were not significantly poorer than IQ-matched controls at identifying the six basic facial expressions of emotion. These results do not support the notion that there is a BAP for basic emotion recognition, in contrast to some previous studies (Palermo et al., 2006; Smalley and

Asarnow, 1990; Wallace et al., 2010). One possible reason for these discrepant findings is that the measure of basic emotion recognition used here was not sensitive enough to detect subtle differences in basic emotion recognition in ASC relatives. Whilst the dependent variable used included a sensitive measure of emotion recognition performance (accuracy-adjusted response time), the KDEF stimuli comprise high intensity, 'full blown' emotions - exaggerated facial expressions - that were relatively easy to identify in non-clinical samples. Making emotional expressions more subtle would have increased task difficulty and may have increased the power to detect subtle differences in emotion recognition ability. A previous study of ASC relatives used the Mind in Eyes test, which requires emotion recognition from just the eye region of the face and involves emotions beyond the basic ones. On the Mind in Eyes test, both mothers and fathers of children with ASC showed deficits (Baron-Cohen and Hammer, 1997). In clinical samples of ASC emotion recognition deficits have also emerged more clearly when using lower intensity stimuli (Law Smith et al., 2010).

A second possible reason for these discrepant findings is that mild difficulties in basic emotion recognition performance may be 'compensated' in parents of children with ASC. Evidence for cognitive compensation has been detected in first-degree relatives using neuroimaging techniques: at a neural level Spencer et al. (2011) found that unaffected siblings of children with ASC, showed reduced neural response (in multiple brain regions including the fusiform face area and superior temporal sulcus) to happy but not fear faces. These neurophysiological differences in siblings were seen despite non-significant differences in performance on the facial emotion recognition task. Understanding what occurs in such examples of 'compensation' will be important in future work.

A third finding from this study relates to adults with ASC. There was a significant sex difference in adults with ASC on the emotion recognition task, females with ASC performing significantly better than males. This contrasts with results on the EQ that did not show significant sex differences in adults with ASC. This suggests that females with ASC may perform better than males with ASC at tests of social cognition, despite having comparably low levels of self-reported empathy.

A number of different interpretations may account for these findings. Females' low self-reported empathy may be more related to difficulties that extend beyond basic emotion recognition which were not analysed here (e.g. more advanced ToM). Alternatively, their low self-reported empathy may reflect higher social expectations on females in the real world. If typical females are expected to be better at empathy than males, this may cause females with ASC to report their empathy problems to a greater degree than males. Finally, these results may reflect greater cognitive compensation in females with ASC. Perhaps as a result of greater social expectations and greater motivation to integrate into social groups, females with ASC work harder to compensate for their problems by developing cognitive strategies to improve their social skills. Thus, females with ASC may have a heightened self-awareness of their social difficulties as a result of being more able than males with ASC to read the emotions of others. This interpretation is consistent with previous studies which find that people with ASC who display stronger intellectual and emotional capabilities perceive themselves as less socially competent than people with ASC who possess less emotional understanding (Capps et al., 1995).

To date, only a small number of studies have investigated behavioural differences between males and females with ASC. Similar to the findings reported here, Lai et al. (2011) found higher levels of autistic traits in females with ASC compared to males on a self-rating scale (the AQ; Baron-Cohen et al., 2001b) but fewer social-communication difficulties on an observational measure (the ADOS; Lord et al., 2002). Further studies are needed to confirm these findings and to test these different explanations.

In addition, the present study replicates previous results showing empathy and emotion recognition in people with ASC. First, empathy difficulties were detected in adults with ASC on the EQ. Like previous studies (Baron-Cohen and Wheelwright, 2004), this study found sex differences in the control group, with typical females reporting significantly higher empathy than males. Likewise, mothers of children with ASC reported significantly higher empathy than fathers of children with ASC. The present study also replicates previous reports of emotion recognition difficulty in adults with ASC (Ashwin et al., 2006; Bölte and Poustka, 2003). However, this study analysed performance on each emotion by taking into account accuracy and response time, and found that adults with ASC have difficulties recognizing both positive (happy) and negative emotions. Difficulties were found across a wider range of basic emotions than reported in previous studies that use smaller sample sizes (Ashwin et al., 2006; Pelphrey et al., 2002). It is possible that very large sample sizes are needed in order to have sufficient power to detect performance differences for specific facial expressions of emotion (e.g. happy and sad expressions).

In addition, many previous studies of facial emotion recognition only examine accuracy as a measure of performance, which is susceptible to ceiling effects and therefore less sensitive to pick up subtle differences in ability. Response time is important because there is strong evidence to suggest that the processing of social information takes longer in individuals with an ASC, perhaps as a result of differences in connectivity patterns within and between structures in the 'social brain' (Brothers, 1990; Isler et al., 2010; Minshew and Williams, 2007). There is also evidence to suggest that milder but similar alterations in brain connectivity can be found in the first-degree relatives of autistic probands (Belmonte et al., 2010; Spencer et al., 2011). Therefore, using a weighted response time measure for social cognition tasks may reveal important subtle differences in cognition between autistic probands, parents and controls, which may not be picked up by accuracy measures alone.

Whilst this study explored group differences on each facial expression of emotion, it is noted that there are also within-subject differences in performance across emotion categories, as shown in Figure 2.4; notably, facial expressions of fear are much harder to recognise than all other categories of emotion. This replicates results from the validation study of the KDEF stimuli database by Goeleven et al. (2008) and has also been found in other studies of emotion recognition (e.g. Gross and Levensen, 1995). A number of possible explanations have been put forward to account for these results; firstly, facial emotion recognition performance may be influenced by participants' experiences identifying different kinds of emotions in daily life, and expressions of fear may be less frequently evoked than other emotions like happiness, anger and sadness, and so are less

easy to recognise. Secondly, fear may be a more complex emotion than other 'basic' emotions using a higher number of facial muscles, making it harder to identify. Thirdly, expressions of fear may overlap strongly with other emotions, particularly surprise, and thus be harder to distinguish compared to other emotions. Further studies are needed that help to tease apart these different possible explanations.

The present study implicates the use of empathy measures as potential endophenotypes for autism. Instead of focusing molecular genetic studies on finding genes associated with clinical diagnoses, studies focusing on endophenotypes may provide measures that are 'upstream' in the causal pathways from genes to clinical diagnosis (Gottesman and Gould, 2003; Chakrabarti et al., 2009). Since both the EQ and KDEF are quantitative measures, these instruments can quantify the heterogeneity in ASC, and may therefore help improve power to detect significant effects, especially for common genetic variants associated with ASC, for which the results have so far been inconsistent (Abrahams and Geschwind, 2008; Freitag et al., 2010; Holt and Monaco, 2011). However, this study suggests that a more subtle test of basic facial emotion recognition is perhaps required for first-degree relatives of children with ASC.

Facial emotion recognition could be a plausible candidate as an endophenotype for ASC. The ability to recognize basic facial expressions appears very early in life (Field et al., 1982; Walker-Andrews, 1997; Walden and Ogan, 1988), is universal across cultures (Ekman and Friesen, 1971) and is acquired in closely related animal species (Darwin, 1872/ 2009). Therefore, it can be hypothesized that this simpler phenotype lies closer to

the genes than the behavioural impairments characterizing ASC using DSM-IV criteria. Likewise, empathy as a trait may be a simpler phenotype than ASC (Chakrabarti et al., 2009).

Currently, only a few studies have tested empathy and emotion recognition as endophenotypes for ASC. For example, a functional MRI study of emotion recognition in children with ASC and their siblings has implicated a neuroimaging endophenotype for responses to happy (versus neutral) faces (Spencer et al., 2011). Likewise, a study investigating the neural correlates of empathising has also suggested that the EQ may constitute a useful endophenotypic parameter for studying ASC (Chakrabarti et al., 2006). Further studies are needed to replicate the results reported here, as well as exploring components of empathy beyond the recognition of basic emotions in people with ASC and their first degree relatives (Decety and Moriguchi, 2007).

There are a number of limitations to acknowledge in this study. First, although all participants in the ASC group reported a clinical diagnosis of ASC, these diagnoses could not be verified because data were collected online. However, Lee et al. (2010) provide evidence to suggest that registering diagnoses of ASC using an online registry of families is accurate. Lee et al. sampled families registered on an online database called the Interactive Autism Network (IAN) and phenotyped 107 children with a registered online diagnosis. 99% of this sample was ASC positive using the ADI-R and 93% was ASC positive on both the ADI-R and ADOS/ expert clinician observation. It is therefore

reasonable to assume that registered online diagnoses for this study are sufficiently reliable, especially in the parent group.

The online study design used in this study also had significant advantages. It enabled collection of much larger sample sizes than those previously on empathy and emotion recognition in people with ASC and their first-degree relatives (Baron-Cohen and Wheelwright, 2004; Baron-Cohen and Hammer, 1997; Bölte and Poustka, 2003; Wallace et al., 2010). Therefore, this study had greater power to detect differences that may not have been picked up in previous investigations looking at similar theoretical constructs. Furthermore, the online measures are completed by people in their own time in the comfort of their own home. This makes the study less stressful than face-to-face testing and may therefore be more valid.

The current study did not include a clinical control group. We cannot therefore exclude the possibility that the lower empathy scores in fathers of children with ASC were due to non-genetic factors associated with caring for a child with special needs. Further studies using a clinical control group are needed to rule out this possibility. Moreover, there were subtle age differences between groups, with parents of children with ASC being somewhat older than the ASC and control groups. Previous studies have reported significantly reduced performance on tests of emotion recognition with increasing age in adulthood (Calder et al. 2003, Montagne et al., 2007). It is therefore important to control for age in data analysis. The sample size was also comparatively small for fathers of children with ASC, but even with this sample size we were able to detect a significant

group effect for fathers of a child with ASC. Power problems due to the relatively small group of fathers are therefore unlikely to play a role. A medium effect size was found using the current sample sizes, and the statistical power (β) was calculated as 0.83 (α = 0.05), which is above recommended levels (Field, 2005).

This investigation used a self-report measure of empathy. Some participants may experience difficulty judging their own empathy, so it would be of interest in future studies to include a measure of empathy rated by others. Ideally, multiple raters would be included to assess empathy (Bartels et al., 2007).

In summary, this study provides support for low self-reported empathy in ASC fathers compared to IQ-matched controls, but no evidence for basic facial emotion recognition difficulties in either parent of a child with ASC. These mild empathy difficulties in ASC fathers confirm earlier studies (Baron-Cohen and Hammer, 1997) and echo the more pronounced deficits found in adults with a clinical ASC diagnosis, who self-reported significantly lower empathy than controls and were also significantly worse at identifying five basic facial expressions of emotion. These findings implicate empathy-related traits as candidate endophenotypes for ASC which could help to elucidate the genetic and biological pathways underlying clinical ASC.

Whilst this study adds to our understanding of ASC endophenotypes by examining two facets of empathy that may be under stronger genetic influence than the clinical ASC phenotype, it has not helped to resolve the inconsistent findings in the BAP research

literature, nor has it furthered our understanding of the modes of genetic transmission that are responsible for the expression of autistic traits and related phenotypes in people with ASC and their relatives. In the next four chapters these issues shall be addressed by exploring the BAP in multiplex versus simplex autism families.

Chapter Three

Exploring the Broader Autism Phenotype in Multiplex versus Simplex Autism Families: General Methods

3.1 Abstract

In the following four chapters the BAP is comprehensively assessed in the unaffected parents of autistic probands from multiplex and simplex autism families, using a battery of performance-based tasks and self-report scales. This current chapter provides an overview of the methodology involved in these forthcoming studies, including: i) a summary of how participants have been recruited and selected, ii) a description of the testing procedure, iii) a summary of the materials used in these studies, iv) a summary of how proband diagnoses have been verified, v) a summary of the criteria used for classifying families into simplex and multiplex groups, and vi) an overall summary of the descriptive characteristics of the ASC parent and proband sample after application of the above criteria. In total, there were 62 families available for analysis; 60 parents from simplex families (30 mothers, 30 fathers) and 64 parents from multiplex families (32 mothers, 32 fathers) (see chapters four to six).

3.2 Introduction

As described at the end of chapter one, the following four chapters empirically examine differences in the expression of autistic traits and related phenotypes in multiplex autism parents, simplex autism parents and controls. The aim of these studies is to reconcile the mixed findings in the BAP research literature by stratifying ASC relatives according to their affiliation to multiplex and simplex family groups, and by discerning whether the BAP is confined to the relatives of multiplex rather than simplex families. This would also support the hypothesis that de novo genetic risk factors of large effect play a stronger role in the aetiology of simplex autism, whilst common, heritable genetic risk factors of weak effect play a stronger role in the aetiology of multiplex autism (Pickles et al., 1995; Sebat et al., 2007; Virkud et al., 2008). This chapter outlines the general methods for these forthcoming studies, starting with participant recruitment and eligibility.

3.3 Participant recruitment and eligibility

The families that participated in the following empirical studies were recruited from multiple sources. Firstly, participants were recruited from a registry of volunteers held on a database that is maintained by the Cambridge University Autism Research Centre. This database contains information on several hundreds of families who have at least one member formally diagnosed with ASC. To be entered into this database, family members must register online by going to the 'volunteers' section of the Cambridge University Autism Research Centre website (www.autismresearchcentre.com).

Secondly, autism charities, support groups and special needs schools around the UK were contacted by email inviting families to take part in the study. In all cases, information sheets were sent to the parents summarising the study and explaining what was involved (see appendix 1). Schools, charities and support groups were contacted in a number of different regions around the country including: Cambridgeshire, Bedfordshire, Kent, Surrey, Lincolnshire, Leicestershire, Gloucestershire, Suffolk, Essex, Surrey and Hertfordshire.

After families had registered an interest in taking part in the project, the mother or father participated in a 10-15 minute telephone interview to check whether the family was eligible. In order to take part in these studies, families had to meet the following criteria:

1. *Parents must have at least one biological child with a formal ASC diagnosis who is able to participate in cognitive and diagnostic assessments.*
2. *The diagnosed child must have at least one full biological sibling;* this criterion was necessary for a family to be classified as multiplex because at least two children must have a clinical diagnosis of ASC. This criterion was also used as a way of increasing the reliability of classifying families as simplex; given that the sibling recurrence rate for autism is around 15-20% (Ozonoff et al., 2011), some simplex families containing only a single diagnosed child may have been classified as multiplex had more children been born in the family. See section 3.7 for further details about the simplex/ multiplex classification criteria.

3. *The diagnosed child must be aged between 6 and 18 years old*; this ensured that all probands completed the same IQ tests.

4. *The diagnosed child must have idiopathic autism (i.e. autism not caused by conditions with a known cause, such as Fragile X and Rett Syndrome);* this criterion is necessary because these studies aim to address the heterogeneity and nature of the genetic mechanisms underpinning cases of autism without a known cause.

5. *Parents must not have a diagnosis of ASC;* it is important to exclude parents who warrant a full diagnosis of ASC for studies into the BAP because the aim of these studies is to assess autistic traits that are occurring below the clinical threshold.

6. *Both mother and father must be willing to participate in the study;* this is because information from both parents was necessary to reliably establish simplex and multiplex status (see section 3.7). This criterion also ensured that both male and female relatives were examined for the BAP; previous studies have detected the BAP in fathers but not mothers (e.g. Happé et al., 2001; De la Marche et al., 2012), whilst one previous study has detected the BAP in mothers but not fathers (Groen et al., 2012).

7. *The family must live within 200-300 miles from Cambridge or Milton Keynes.*

As a result of these criteria, a number of families were ineligible and so had to be excluded from these studies. Reasons for ineligibility included: (1) divorced parents (and only one parent willing to participate in the study), (2) fathers who declared that they did not wish to take part in the study, (3) proband ages below the minimum of 6 years, (4) parents who declared that they had ASC and (5) families that were located beyond 300 miles from Cambridge or Milton Keynes.

74 families in total passed these eligibility criteria. Table 3.1 shows that these families were located in 20 different counties across the United Kingdom. The highest numbers of families were located in Suffolk (12%), Hertfordshire (12%) and Essex (11%) (see Table 3.1).

Table 3.1: *A summary of the geographical locations of recruited families.*

Location	N families tested	% of sample
Suffolk	9	12.2
Hertfordshire	9	12.2
Essex	8	10.8
Lincolnshire	6	8.1
Kent	6	8.1
Cambridgeshire	5	6.8
Norfolk	5	6.8
Leicestershire	4	5.4
Greater London	4	5.4
Surrey	4	5.4
Bedfordshire	3	4.1
Lancashire	2	2.7
Buckinghamshire	2	2.7
Avon	1	1.4
Warwickshire	1	1.4
Middlesex	1	1.4
Northamptonshire	1	1.4
Derbyshire	1	1.4
Yorkshire	1	1.4
Worcestershire	1	1.4

Finally, there were two remaining criteria that had to be met in order for families to be included in the data analyses for studies reported in chapters four to six:

8. *One proband from each family must meet research diagnostic criteria* (see section 3.6).

9. *Families must meet simplex/ multiplex classification criteria* (see section 3.7).

3.4 Procedure

If families met the eligibility criteria 1-7 outlined in section 3.3 then a date and time was arranged to test the family members. Families could choose to be tested at home or in testing rooms at either the Open University or the Cambridge University Autism Research Centre. An information package was sent to the eligible families containing a confirmatory letter of the testing time/ location, maps of the testing centre (if applicable) and the self-report/ parental-report questionnaires (see section 3.5), which had to be completed prior to the home visit. Parents were also given the option of completing these questionnaires online by registering with the Cambridge University Autism Research Centre (www.autismresearchcentre.com). On the testing day, E.S. administered the 3Di parental interview, Mind in eyes and KDEF tasks using a laptop. E.S. also administered the ADOS-G, whilst a research assistant administered the IQ tests and the EFT (see section 3.5 for detailed descriptions of the materials and appendix 2 for a copy of the testing schedule). The testing time was approximately 4-5 hours per family.

3.5 Materials

3.5.1 Selection of measures to assess the BAP; rationale

Parents completed four self-report questionnaires and three performance-based tasks in total with the aim of obtaining a full picture of the BAP whilst keeping testing time to a

minimum. As well as including a questionnaire measure of autistic traits (the AQ; Baron-Cohen et al., 2001b), the tasks and self-reports used in this study were associated with two related psychological constructs: empathy and systemising[17] (Baron-Cohen, 2002; and see chapter two). The empathy measures were used because there are a number of studies, described in chapter one, that suggest that people with ASC and their relatives perform poorly on tasks involving empathy/ social cognition, for example the Mind in Eyes task which tests people's ToM ability (Baron-Cohen et al., 2001a, Dorris et al., 2004). Likewise, empathy difficulties were found in people with ASC and fathers of autistic probands in chapter two. There are also a number of studies suggesting that people with ASC perform well on tasks related to systemising, for example the EFT that tests perceptual attention to detail (Jolliffe and Baron-Cohen, 1997, Pellicano et al., 2006). A small number of studies have also found superior performance on the EFT in first-degree relatives (e.g. fathers; Happé et al., 2001; see chapter one, section 1.3.2.3). Therefore, the following studies also aimed to explore whether these cognitive strengths could be found in relatives of people with ASC, after stratifying families according to simplex and multiplex criteria (see section 3.7 and chapters four to six).

Parents also completed an additional self-report measure (the Adult Self Report Form; Achenbach and Rescorla, 2003) that measures traits consistent with other psychiatric conditions, such as depression and anxiety. This was included because the research literature on the BAP suggests that relatives of people with ASC may show signs of other

[17] For a definition and description of empathy see chapter two, section 2.2. Systemising is here defined as 'the drive to analyse the variables in a system, to derive the underlying rules that govern the behaviour of a system (and)..the drive to construct systems.' (Baron-Cohen, 2002; Baron-Cohen et al., 2003)

psychiatric conditions, such as depression, anxiety disorder and Obsessive Compulsive Disorder (see chapter one, section 1.3.3).

A full diagrammatic summary of measures used in this study is provided in Figure 3.1 on page 144. Further information about these measures is described in the next section.

Assessing the BAP: self-report scales

3.5.2 *The Autism-Spectrum Quotient (AQ)*

The Autism-Spectrum Quotient (AQ) is a self-report or parent-report questionnaire, designed to quantitatively measure autistic traits in adults and children with ASC as well as in the general population (Baron-Cohen et al., 2001b; Auyeung et al., 2008; Hoekstra et al., 2008; see appendix 3 for a copy). The AQ contains 50 items in total that assesses DSM-IV criteria symptoms covering the 'triad of impairments' (social skills, social communication and restricted interests) as well as assessing cognitive-behavioural features including attention to detail and lack of imagination. Participants must rate each item using a 4 point Likert Scale (1 = 'definitely agree', 2 = 'slightly agree', 3 = 'slightly disagree', 4 = 'definitely disagree' e.g. 'I find myself more strongly drawn to people than to things'). Using the scoring system provided by Hoekstra et al. (2008), the maximum achievable score for the questionnaire is 200 (full endorsement of statements describing autistic traits) while the minimum score is 50 (no autistic traits). Studies have demonstrated that the AQ has reasonable construct and face validity, good inter-rater

reliability and good test-retest reliability (r = 0.7; Baron-Cohen et al. 2001; r = 0.8; Hoekstra et al. 2008). The AQ is also capable of differentiating ASC groups from typically developing and clinical control groups (Baron-Cohen et al., 2001b; Woodbury-Smith et al., 2005; Hoekstra et al., 2008; Auyeung et al., 2008). Sex differences have also been reported in studies on the AQ, with males scoring significantly higher than females (e.g. Baron-Cohen et al. 2001b; Hoekstra et al., 2008). A number of factor analyses have been carried out on the AQ (e.g. Austin, 2005; Hoekstra et al., 2008; Hurst et al., 2007) with all studies converging on at least 2 factors e.g. a higher order 'social interaction' factor and an 'attention to detail' factor (Hoekstra et al., 2008).

It was also necessary to briefly screen siblings of the proband for autistic traits, and so parents also completed the child version of the Autism-Spectrum Quotient (Auyeung et al., 2008) for the proband's siblings. Sibling scores on this measure were used in the simplex/ multiplex classification criteria for chapters four to six (see section 3.7).

3.5.3 *The Empathy Quotient (EQ)*

The Empathy Quotient (EQ) is a self-report or parent-report questionnaire, designed to quantitatively measure a person's empathy (see chapter two; and appendix 4 for a copy of the questionnaire). The EQ contains 40 items with a 4 point Likert scale for each item: (1 = definitely agree', 2 = 'slightly agree', 3 = 'slightly disagree', 4 = 'definitely disagree' e.g. 'it upsets me to see an animal in pain'). The maximum achievable score for the questionnaire is 80 whilst the minimum score is 0. Adults with Asperger Syndrome/

High-Functioning Autism (HFA[18]) score significantly lower on the EQ compared to typically developing, age-matched controls (Baron-Cohen and Wheelwright, 2004). In the general population, women score significantly higher than men (Baron-Cohen and Wheelwright, 2004). The EQ has demonstrated good validity and excellent test-retest reliability (Baron-Cohen and Wheelwright, 2004; Lawrence et al., 2004; see chapter two, section 2.3.2). Whilst previous factor analyses of the EQ suggest there might be three underlying factors that the questionnaire is tapping into (Lawrence et al., 2004; Berthoz et al., 2008), a more recent examination of the dimensionality of the EQ using a Rasch analysis suggests that the questionnaire has a unidimensional structure (Allison et al., 2011).

3.5.4 *The Systemising Quotient-Revised (SQ-R)*

The Systemising Quotient-Revised (SQ-R) is a self-report or parent-report questionnaire designed to quantitatively measure systemising (see appendix 5 for a copy). This psychological construct is consistent with a number of clinical descriptions (e.g. the tendency to collect and organise items, a strong preference for constructional and vehicle toys etc.) and has been hypothesised to underlie the non-social clinical symptoms of ASC (Baron-Cohen, 2002). The SQ-R consists of 75 items with responses on a Likert Scale ranging from 'strongly agree' to 'strongly disagree' (e.g. 'I can easily visualise how the motorways in my region link up'). The maximum score for the questionnaire is 150 whilst the minimum score is 0. Studies demonstrate that the SQ-R differentiates ASC groups from typically developing controls; ASC groups score significantly higher SQ-R

[18] HFA is a term used to describe people with autism who do not have intellectual disability (IQ > 70).

scores demonstrating an intact or superior tendency to systemise (Baron-Cohen et al., 2003).

Assessing the BAP: performance-based tasks

3.5.5 *Reading the Mind in the Eyes Task ('Mind in Eyes')*

The Reading the Mind in the Eyes Task ('Mind in Eyes') is a performance-based measure that assesses people's ability to deduce the mental states of others from looking at images of the eye region of the face only. 36 photographs of eyes are shown for the adult version (Baron-Cohen at al., 2001a). Participants are asked to choose the correct word from a choice of four that best describes what the person in the photograph is feeling or thinking. Therefore, this test assesses people's 'cognitive empathy' abilities, that is, the ability to infer the subtle mental states of others using limited social information (the eyes region of the face). These mental state terms go beyond 'basic' emotional states (cf. Ekman and Friesen, 1971) and include states requiring the attribution of an intention or belief (e.g. 'interested', 'cautious', 'thoughtful'). Studies have demonstrated that people with ASC are significantly impaired at this task compared to non-clinical control groups (Baron-Cohen et al., 1997, 2001a). In addition, these findings have been extended to include relatives of people diagnosed with ASC (e.g. Baron-Cohen and Hammer, 1997; Dorris et al., 2004). Sex differences have also been reported for this task, with females scoring higher than males on average (Baron-Cohen et al., 2001a).

3.5.6 *The Karolinska Directed Emotional Faces Task (KDEF)*

The Karolinska Directed Emotional Faces Task (KDEF) assesses people's ability to recognise basic emotions from pictures of facial expressions (see chapter two). It contrasts with the Mind in Eyes task where social information available to participants is severely restricted and the choice of mental state terms provided are more complex. As described in chapter two, the KDEF contains 140 photographs of people's faces expressing one of six 'basic' emotions (happy, sad, angry, afraid, disgusted and surprised) as well as a neutral expression. Participants must choose which of the seven options fits the emotion being expressed in each photograph. The stimuli used in the KDEF have been well validated on emotional content, intensity and arousal (Goeleven et al., 2008). The study reported in chapter two has demonstrated that adults with ASC perform significantly worse on this test compared to IQ-matched controls, including the recognition of negative and positive emotions. This supports two previous studies demonstrating basic emotion recognition impairments using the KDEF in people with ASC (Ashwin et al., 2006; Lai et al., 2012). The study described in chapter two did not report milder difficulties on this test in the first-degree relatives of autistic probands and there have been no previous studies that have used this task on the relatives of autistic probands. However, a similar task was used by Bölte and Poustka (2003) to assess social cognition in the relatives of probands from simplex and multiplex autism families; multiplex relatives were reported as scoring significantly lower on this emotion recognition task than simplex relatives. The task involved the same 6 basic emotions and neutral expression used in the KDEF. Therefore, these results suggest that basic emotion

recognition difficulties may be found in ASC relatives using the KDEF if the sample is first stratified into multiplex and simplex groups.

3.5.7 *The Embedded Figures Task (EFT)*

The Embedded Figures Task (EFT) is a visual search task where participants must detect a hidden simple shape embedded within a complex larger figure (Witkin et al., 1971). There are 12 figures and simple shapes in total, which vary in terms of difficulty detecting the embedded shape. A number of studies have shown that individuals with ASC and their relatives have a faster mean response time (RT) on this task compared to control groups, especially in male probands and fathers (Jolliffe and Baron-Cohen, 1997; Happé et al., 2001; but see White and Saldaña, 2011). In the general population, response times on this task correlate with the AQ, such that greater endorsement of statements consistent with autistic traits (higher AQ score) are associated with faster performance at no significantly reduced cost to accuracy (Grinter et al., 2009). These studies therefore suggest that people with ASC and people with high autistic traits may be superior in local or piecemeal processing of visual stimuli and may therefore perform well on tasks that require strong attention to detail.

Assessing the BAP: other psychiatric conditions

3.5.8 *The Adult Self-Report Form (ASR)*

The Adult Self-Report Form (ASR; Achenbach and Rescorla, 2003) is a self-report questionnaire that assesses traits consistent with other mental health problems (see appendix 6 for a copy). It consists of 126 items that examine traits consistent with DSM-defined psychiatric conditions, including depression, anxiety, avoidant personality, Attention Deficit/ Hyperactivity Disorder and anti-social personality disorder. The same items can also be split into several syndrome scales, including 'anxious/ depressed', 'withdrawn', 'somatic complaints', 'thought problems', 'attention problems', 'aggressive behaviour', 'rule breaking behaviour' and 'intrusive' behaviour. Test-retest reliability for the ASR is reported as high, with Pearson test-retest correlation scores above 0.8 for most scales (all significant at $p < 0.01$; Achenbach and Rescorla, 2003). The internal consistency of the items comprising each scale range from 0.51 to 0.97 (Cronbach's alpha). Finally, cross-informant correlations for each scale range from 0.3 to 0.79 (all $p < 0.001$; Achenbach and Rescorla, 2003).

Assessing General Cognitive Functioning

3.5.9 *The Raven's Progressive Matrices*

The non-verbal intellectual abilities of parents and proband were assessed using the Raven's Progressive Matrices (Raven, 2000). This assessment is a widely administered measure of non-verbal IQ and can be reliably used on both adults and children. Parents

and proband took the 'standard' version of the matrices, which is designed for people over 7 years of age. In cases where the proband was younger than 7 years of age and/ or had severe learning disabilities, the 'coloured' version was used. The Standard Progressive Matrices can be administered to groups allowing the parents to be tested on this measure at the same time.

The Standard Progressive Matrices is a pencil and paper test containing 60 items. For each item, participants must look at a pattern with a piece missing and identify the correct piece from a choice of six that fits the pattern. The test takes approximately 40 minutes to complete.

3.5.10 *The Raven's Mill-Hill and British Picture Vocabulary Scales*

Verbal intellectual functioning was also measured in the parents and proband. Parents were examined using the Mill-Hill Vocabulary Scale (Raven, 2000), which is designed for use in tandem with the Raven's Progressive Matrices. Participants were asked to complete two sections: in the first section, participants wrote down the meaning of 33 words. In the second section, participants selected one word from a group of six that was closest in meaning to a word displayed in bold type.

Probands completed a different verbal IQ measure, called the British Picture Vocabulary Scale-II (BPVS-II; Dunn et al., 1997). The BPVS is administered by giving participants a choice of 4 pictures and asking the participant to point to the picture that describes the

meaning of a word. Items are split into sets of 12; at the beginning of the test, the participant must answer 10 items correct before continuing to the next set of items. Once 8 or more wrong answers are given the test is stopped, the participant's score is counted and their verbal IQ is calculated. The BPVS-II assesses people's receptive vocabulary and is preferable to other verbal IQ measures for children with ASC because it can assess their verbal abilities without necessitating a verbal response and appeals to the tendency for individuals with ASC to think using visual representations (e.g. Grandin, 1995).

Verifying proband diagnosis

3.5.11 *The Developmental, Dimensional and Diagnostic interview-short (3Di-short) and 3Di 'family' section*

The 3Di is a computerized parental interview about a child's developmental history (Skuse et al., 2004), comparable to the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). However, unlike the ADI-R, the 3Di assesses the severity of ASC along multiple dimensions of impairment. In doing so, autism symptomatology is conceptualised as occupying the extreme end of a continuum that merges into the 'normal-range' of behaviours. The 3Di-short is a shortened version of the full clinical interview, taking approximately 45-60 minutes to complete (Santosh et al., 2009). It is composed of 53 questions that are designed to rapidly assess autistic symptomatology within the three core domains of impairment: (1) reciprocal social interaction skills, (2) use of language and other social communication skills and (3) repetitive/ stereotyped behaviours and routines. It generates automatic reports of autism symptomatology

covering 4 subscales (social reciprocity, communication, non-verbal communication and repetitive behaviours). Scores on each subscale must exceed a given threshold to achieve clinical significance. In addition to this rapid assessment of ASC, some additional questions were asked about the proband's siblings and parents' own development in order to further establish whether other family members display features consistent with the BAP (see chapter six); these were taken from the 'family' section of the 3Di interview. The 3Di interview has demonstrated strong inter-rater reliability and test-retest reliability (Skuse et al., 2004). A measure of inter-rater reliability was also obtained on the 3Di-short for the current sample, with a second researcher independently rating 19 3Di-short audiotaped interviews. Correlation coefficients were extremely high on all four subscales (social reciprocity; $r = 0.92$, $p < 0.001$; communication; $r = 0.97$, $p < 0.001$; non-verbal communication; $r = 0.96$, $p < 0.001$; repetitive behaviours; 0.92, $p < 0.001$), and so inter-rater reliability was very strong for this assessment.

3.5.12 *The Autism Diagnostic Observational Schedule-Generic (ADOS-G)*

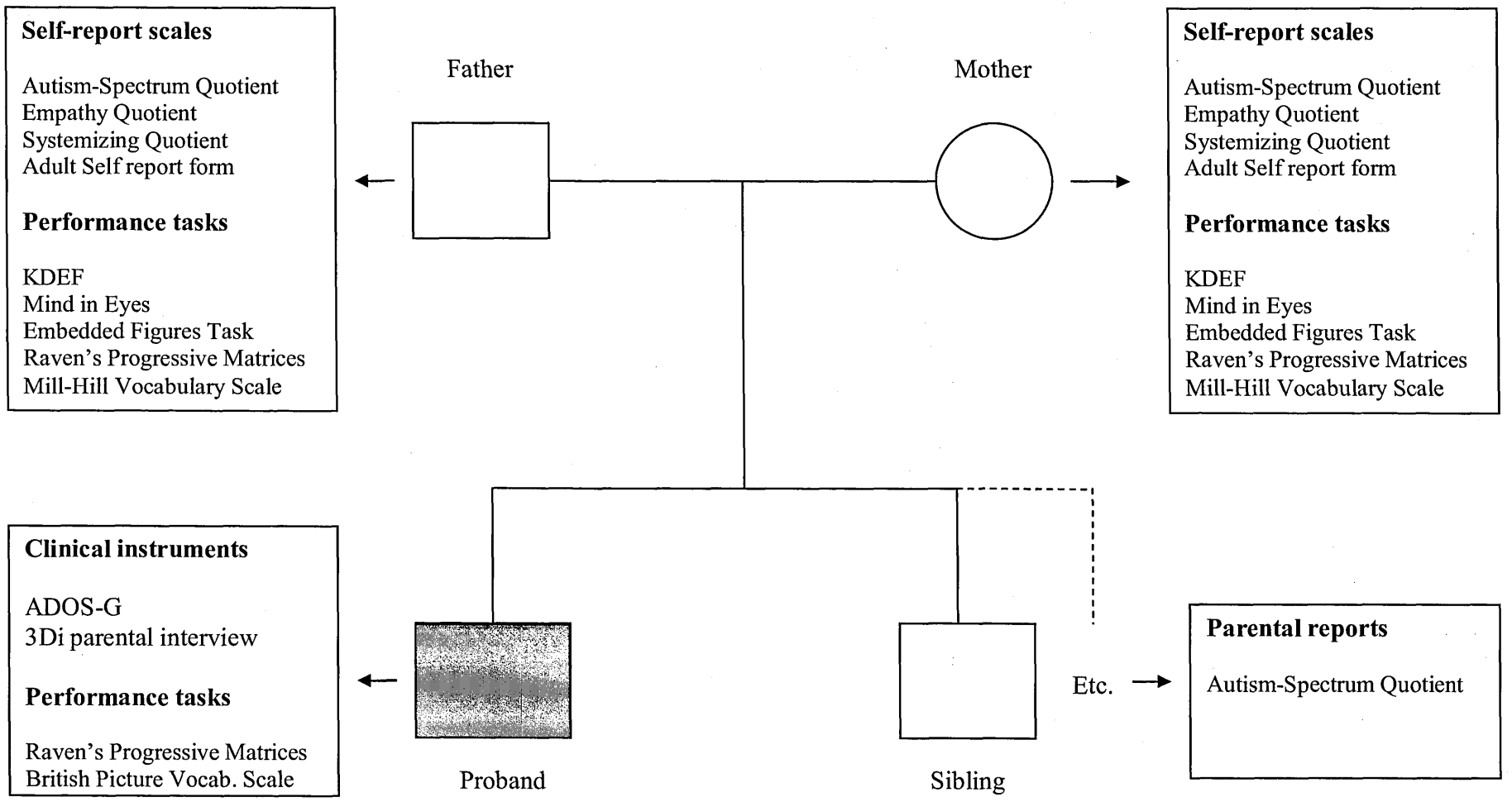
The Autism Diagnostic Observational Schedule-Generic (ADOS-G; Lord et al., 2000) is a semi-structured observational assessment designed to assess 4 domains of functioning: communication, reciprocal social interaction, imagination and stereotyped behaviours and restricted interests. The examiner must choose one of four different 'modules' which are designed to assess these areas of function in individuals at different stages of development and verbal competency. In each module, the examiner asks the individual to

participate in a variety of activities such as reading a storybook, making up a story using objects or describing a picture. For the more advanced modules, the examiner also asks questions about relationships and emotions, as well as initiating conversations and encouraging the individual to reciprocate by initiating conversations of their own. Each module takes approximately 40-50 minutes to complete, after which the examiner rates the individual's behaviour on the different domains of functioning listed above. Scores for two of these domains (communication and reciprocal social interaction) are combined to generate an ADOS-G score that must be over a threshold to achieve clinical significance. In contrast to the parental 3Di interview, the ADOS-G gives researchers the chance to formally assess autism symptomatology face-to-face for a limited time period. Studies have demonstrated that the ADOS-G has strong inter-rater reliability and good test-retest reliability within each behavioural domain (e.g. intraclass correlations; social interaction = 0.78 and communication = 0.73; Lord et al., 2000).

Table 3.2: *A summary of measures used and their properties*

Type of measure	Name of measure	Characteristic(s) measured	Number of items	Item information	DV(s) used	Test-retest reliability	Other information
Self-report questionnaire	AQ	Self-rated autistic traits	50	Respond using 4 Point Likert scale	Summed total score and 2 factor subscale scores	Good (r = 0.7-0.8)	Reasonable construct and face validity, good inter-rater reliability.
	EQ	Self-rated empathy	40	Respond using 4 Point Likert scale	Summed total score	Excellent (r = 0.97)	Good construct validity, inter-rater reliability not reported.
	SQ-R	Self-rated systemizing	75	Respond using 4 Point Likert scale	Summed total score	Not reported	Construct validity and inter-rater reliability not reported.
Performance-based task	Mind in Eyes	Mental state perception	36	Multiple choice; 4 mental state terms presented	Accuracy	Not reported	Good construct and ecological validity.
	KDEF	Basic facial emotion recognition	140	Multiple choice; 7 emotion labels presented	Response time and accuracy	Good (r = 0.88)	Stimuli validated on emotional content and intensity; good ecological validity.
	EFT	Perceptual attention to detail	12	Item completed when participant correctly traces around shape	Response time and accuracy	Not reported	Good face validity; construct validity not reported.

Figure 3.1: *A Schematic representation of measures used for empirical chapters four to seven*



3.6 Verifying diagnoses of ASC for research purposes

Before data analysis was conducted, it was important to collect evidence that confirmed that the probands warranted a research diagnosis of ASC (see sections 3.5.11 and 3.5.12). The criteria used for verifying ASC diagnoses are provided in the flowchart in Figure 3.2. In summary, probands (N = 32) who met full criteria for ASC on both the 3Di-short interview and the ADOS-G were included in subsequent data analyses. To meet full clinical criteria for ASC on the 3Di-short, probands had to exceed a threshold score on the reciprocial social interaction symptom domain of the interview as well as exceed a threshold score on either the communication symptom domain or the repetitive/ stereotyped behaviours and interests symptom domain of the interview (see Table 3.3). To meet full clinical criteria for ASC on the ADOS-G, probands had to exceed a threshold score on the reciprocal social interaction symptom domain and the communication symptom domain as well as exceed a threshold score when the reciprocal social interaction and communication symptom domain scores are combined (see Table 3.4). Probands (N = 7) who did not meet full clinical criteria for ASC on both the 3Di-short and the ADOS-G were excluded from subsequent analyses (along with the proband's parents) (N = 7 families). As a minimum requirement, probands (N =35) had to meet full clinical criteria on either the 3Di-short or ADOS-G. 3 probands were identified who did not show evidence of clinical impairment on any of the symptom domains on the ADOS-G (as described above and in Table 3.4). These 3 probands were second reviewed by a senior researcher at the Cambridge Autism Research Centre and a consensus was

reached as to whether a research diagnosis was warranted. If not, then they were excluded from subsequent analyses along with the probands' parents. A consensus was reached that two of these probands displayed sufficient evidence to warrant a research diagnosis, whilst the other proband did not and so was excluded from subsequent data analysis along with the proband's parents.

Table 3.3: *A summary of the clinical criteria for ASC on the 3Di-short*[19]

SYMPTOM DOMAIN 1	**SYMPTOM DOMAIN 2**	**SYMPTOM DOMAIN 3**	**Diagnostic category**
Is RSI score over clinical threshold (11.5)?	Is communication score over clinical threshold (8.0)?	Is RBI score over clinical threshold (5.0)?	
Yes	Yes	Yes	Autistic disorder/ Asperger Syndrome
Yes	No	Yes	Atypical autism
Yes	Yes	No	Atypical autism

Table 3.4: *A summary of the clinical criteria for ASC on the ADOS-G*[20]

SYMPTOM DOMAIN 1	**SYMPTOM DOMAIN 2**	
Is RSI score over clinical threshold?	Is communication score over clinical threshold?	Is RSI + communication score over clinical threshold?
Yes	Yes	Yes

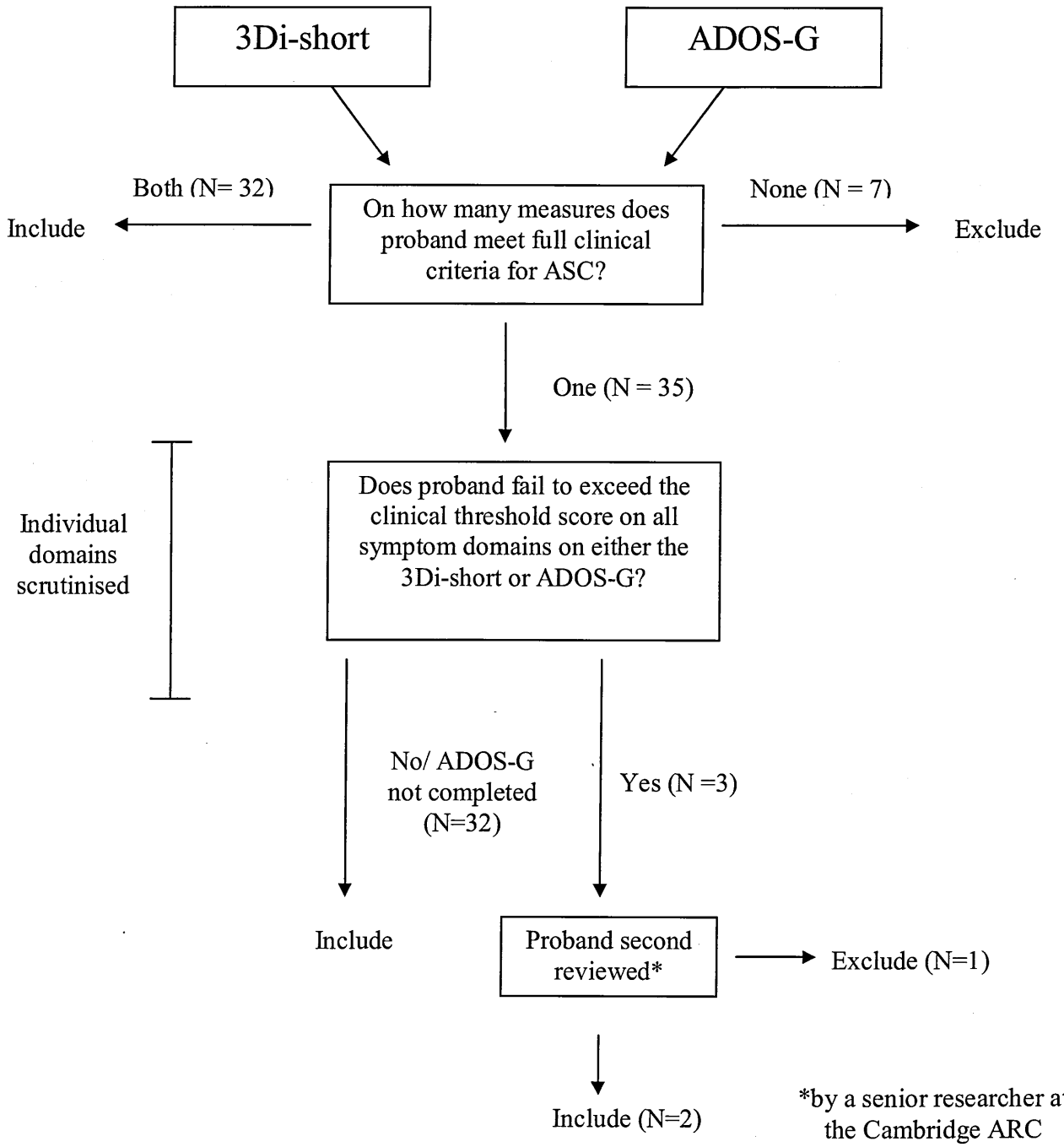
Some previous studies have only used a parental interview about the proband's developmental history to verify proband diagnoses rather than combining a clinical observational assessment (e.g. ADOS) with the parental interview (e.g. ADI-R; Lai et al.,

[19] RSI: Reciprocal Social Interaction; RBI: Repetitive/ Stereotyped Behaviours and Interests
[20] RSI: Reciprocal Social Interaction

2011). However, the above criteria was preferred to this strategy because it gives equal weight to the ADOS-G and the 3Di-short and allows for a degree of convergent clinical agreement on both measures. Relying on either clinical instrument alone may be unreliable, since both instruments have their strengths and their weaknesses; for example, the ADOS-G allows the examiner to assess ASC symptomatology first-hand but there are severe time constraints and the examiner may not observe more subtle symptoms that are detected over a longer time period or in different/ more complex social settings. The parental interview about developmental history largely overcomes these issues because the parent/ caregiver has observed the proband throughout his/ her development and in a number of different social environments. However, the interview relies on a secondary source (i.e. the parent/ caregiver) for information and thus is completely reliant on the accuracy of the informant's observations. Therefore it is here argued that, as far as possible, it is necessary to involve both clinical instruments in a proband diagnosis verification procedure, rather than relying on the parental interview or observational assessment only.

So in summary, 8 families were excluded from future data analyses because there was not sufficient evidence that the proband warranted a research diagnosis of ASC. Thus, a total of 66 families could be used in data analysis for studies on the BAP (see chapter seven) before application of the simplex/ multiplex classification criteria (see section 3.7).

Figure 3.2: *Flowchart displaying proband research diagnosis criteria.*



3.7 Simplex/ Multiplex Classification Criteria

In chapters four to six, families are classified into those containing a single case of ASC (simplex families) and those containing multiple cases of ASC (multiplex families). The criteria for classifying families into simplex and multiplex are summarised in Figure 3.3 (page 152). These criteria aim to be an improvement upon previous studies that have examined the BAP in simplex and multiplex families. Some of these previous studies had severe methodological limitations e.g. Virkud et al. (2009) included in their analysis siblings from multiplex families who had been diagnosed with ASC, thus inflating the difference in scores on their measure of autistic traits in the 'unaffected' relatives of multiplex versus simplex autism families (Hoekstra and Wheelwright, 2009). Other studies did not clarify whether 'affected' first-degree relatives were removed from the samples before data analysis (e.g. Losh et al., 2008). In this project, steps were taken to prevent the inclusion of first-degree relatives who had (or may have warranted) a full diagnosis of ASC; parents took part in a telephone interview where they were asked if they had a formal diagnosis of ASC. If either parent had a diagnosis, then they were considered as ineligible for this project and so were prevented from participating.

The inclusion criteria for simplex families were as follows:

(1) Families must contain one child with a verified formal ASC diagnosis (see section 3.6) plus one or more siblings without a reported diagnosis of ASC.

(2) The unaffected sibling(s) must be over three years of age and score at or under a screening threshold on the AQ (75; Auyeung et al., 2008): it is possible that some of the unaffected siblings of children with ASC in provisional simplex families may warrant a diagnosis of ASC but have not been detected yet, which again would affect a family's status as being 'simplex'. Unaffected siblings needed to be over three years of age, which is the minimum age a child can be diagnosed with ASC. Siblings who exceeded the AQ threshold cut-off score were considered to be at an increased risk of having ASC and therefore not considered reliable enough to be classified as a simplex family.

(3) Families must not contain any members in the extended family with formal ASC diagnoses; this ensured that there was only a single case of clinical ASC in both the 'nuclear' and extended family. This criterion was an improvement upon some previous studies, which did not take into account diagnoses in the extended family (e.g. Losh et al., 2008; Virkud et al., 2009).

The inclusion criteria for multiplex families were as follows:

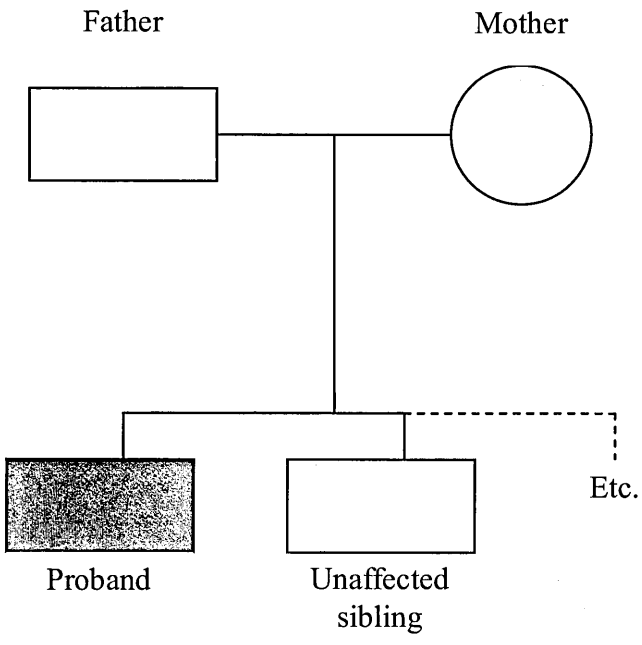
(1) Families must contain at least two children with a formal diagnosis of ASC.

(2) One of the autistic probands must have their diagnosis verified using the 3Di-short and ADOS-G (see section 3.6), whilst at least one other diagnosed proband must score over a screening threshold score on the AQ (75; Auyeung et al., 2008); whilst ideally all autistic probands in the family would have had their diagnosis verified more comprehensively using the 3Di-short and ADOS-G, this would have been too time-

consuming. The criterion described here at least ensured that firstly two or more diagnoses in the family were verified, and secondly that time was available for families to complete the cognitive tasks and self-report scales described in section 3.5 and chapters four to seven.

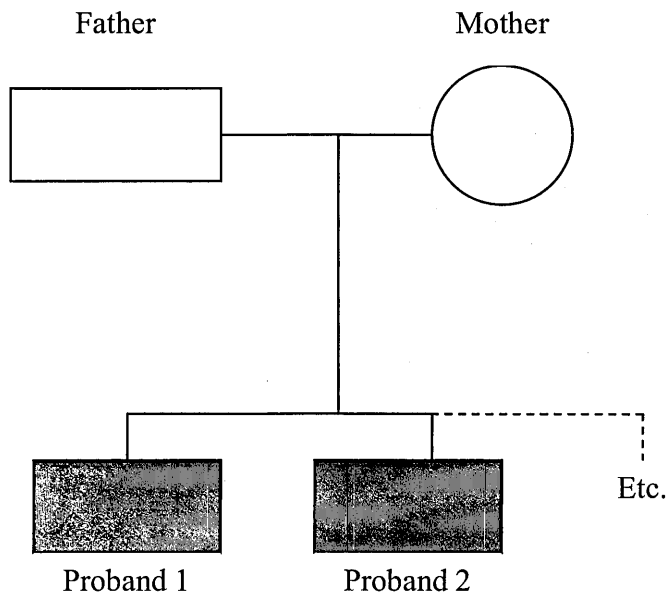
After applying the criteria outlined above and in Figure 3.3, a total of four families had to be excluded from data analyses. These included three provisional simplex families that contained 'unaffected' siblings with AQ scores above the screening threshold and one provisional simplex family that did not contain a full biological sibling. Therefore, a total of 62 families were analysed; 30 simplex families and 32 multiplex families (see chapters four to six).

Figure 3.3: *Schematic representation of simplex/ multiplex classification criteria.*



Criteria

1. Families must contain one child with a formal verified ASC diagnosis plus one or more full siblings without a diagnosis.
2. The 'unaffected' siblings must be over three years of age and score at or under a screening threshold on the AQ (75).
3. Families must not contain members in the extended family with formal ASC diagnoses.



Criteria

1. Families must contain at least two full siblings with a formal ASC diagnosis.
2. One diagnosed full sibling must have their diagnosis verified using ADOS and 3Di-short and at least one other diagnosed full sibling must score over a screening threshold score on the AQ (75).

3.8 Participant Characteristics

3.8.1. ASC Parent Sample

A total of 124 parents (62 mothers, 62 fathers) were available for data analyses after applying all the eligibility criteria listed in section 3.3. Descriptives for the ASC parent sample are provided in Table 3.5, including mean age and mean verbal and non-verbal (performance) IQ. Simplex parents had a very similar mean age to multiplex parents. Parents scored slightly below the normative mean for non-verbal IQ, and were very similar to population norms for verbal IQ. There were no significant differences between multiplex and simplex parents on any of these measures (all $p > 0.05$).

Table 3.6 displays the educational level of the ASC parent sample and compares it to a normative adult sample in the UK reported by the Department of Education (see appendix 7). Table 3.6 shows that the parents tested had a somewhat higher educational level than a representative sample from the UK population; 94% of the parent sample had an NQF Level 2 qualification or higher compared to 77% in the representative sample. Likewise, a higher percentage of the ASC parent sample had an NQF Level 3 and NQF Level 4 qualification or higher compared to the normative sample. Therefore, although verbal and non-verbal IQ scores in the ASC parent sample were similar to population means, higher educated parents seem to be slightly over represented in this sample. Table 3.6 also displays the educational level of ASC parents split into multiplex and simplex

groups; the differences between multiplex and simplex parents were not significant ($p > 0.05$).

Table 3.5: ASC *Parent sample descriptives.*[21]

	All Parents			Multiplex Parents			Simplex Parents		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Age (exact)	123	44.7	6.3	63	44.5	5.3	60	44.9	7.3
PIQ	122	97.2	11.3	62	96.9	11.4	60	97.6	11.1
VIQ	122	99.4	13.6	62	98.3	13.9	60	100.4	13.4

Table 3.6: *Parent educational level versus a sample from the general population.*[22]

Educational qualification	ASC Parents (N = 118)	Multiplex Parents (N = 61)	Simplex Parents (N = 57)	Normative Sample (n = 35, 879)
% NQF Level 2 or above	94	92	97	77
% NQF Level 3 or above	70	67	74	58
% NQF Level 4 or above	59	54	63	37

3.8.2 Proband Sample

Descriptives for the proband sample are displayed in Table 3.7, including mean age, verbal and non-verbal (performance) IQ. The sample was comprised of 62 probands in total. Mean non-verbal IQ was slightly above the normative average whilst verbal IQ was very close to population norms. Multiplex probands had a very similar mean age to simplex probands, and the non-verbal and verbal IQ scores were similar to population means in both groups. There were no significant differences on these measures between groups (all $p > 0.05$). 27 out of the 30 probands from simplex families were male (male:

[21] PIQ: Performance IQ; VIQ: Verbal IQ. NB: a small number of parents from multiplex families did not complete the IQ tests or report their age.

[22] NQF: National Qualifications Framework. NB: 6 ASC parents did not report their educational qualifications. For further details about the normative sample and the educational qualification categories, see appendix 7.

female sex ratio = 9:1) compared to 25 out of 32 probands from multiplex families (male: female sex ratio = 3.6:1). This difference was not statistically significant ($p = 0.30$). Tables 3.8 and 3.9 and Figure 3.4 summarise the results from the two clinical instruments (3Di-short and ADOS-G) for this proband sample. Mean 3Di scores were above clinical thresholds on each subscale in both simplex and multiplex proband groups and there were no significant group differences in scores on each 3Di subscale (all $p > 0.05$). Likewise, Table 3.9 shows that median ADOS-G scores on each subscale for each module were above the clinical cut-offs.

Figure 3.4 displays the percentage of probands with each diagnostic sub-category; the first chart summarising the sub-categories reported by the proband's parents (i.e. the clinical diagnosis), the second according to the clinical instruments (i.e. the research diagnosis, based on the 3Di-short interview and the proband's PIQ score). These show that the majority of participants were diagnosed with High-Functioning Autism or Asperger Syndrome. A considerably larger number of probands were given a research diagnosis of atypical autism[23] compared to reported clinical diagnoses (31% vs. 2%). Furthermore, fewer probands were given a research diagnosis of Asperger Syndrome (37% vs. 50%; see Figure 3.4). These differences between the diagnostic categories reported by clinicians and the research diagnostic categories obtained here likely reflects the lack of consistency and reliability of assigning clinical catgeories of ASC across multiple locations of clinical sites, as described by Lord et al. (2011).

[23] Atypical autism is taken from the ICD-10 classification system of mental and behavioural disorders (WHO, 1993) and is widely considered to be commensurate with PDD-nos. This diagnostic sub-category is used rather than PDD-nos in the 3Di assessment.

Taken together, these results suggest that any differences that may be identified between parents from simplex and multiplex autism families in chapters four to six are unlikely to be caused by differences in the probands of these families. We now turn our attention to chapter four, which is the first to examine the BAP in multiplex versus simplex autism parents and controls.

Table 3.7: *Proband sample descriptives.*[24]

	All probands				Multiplex probands				Simplex probands			
	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range
Age (years)	62	12.2	3.0	7.0-18.7	32	12.3	2.8	8.1-18.5	30	12.1	3.2	7.0-18.7
PIQ	60	103.5	21.1	55-145	31	105.3	15.8	75-140	29	101.5	25.6	55-145
VIQ	55	99.7	25.9	43-160	28	100.6	24.3	51-160	27	98.7	27.8	43-156

Table 3.8: *3Di parental interview descriptives.*[25]

3Di Subscales (minimum score with clinical significance)	**All probands** (N= 62)		**Multiplex probands** (N = 32)			**Simplex probands** (N = 30)		
	Mean	SD	Mean	SD	Range	Mean	SD	Range
RSI (11.5)	15.5	3.7	16.0	3.9	9.2-24.8	15.0	3.6	6.7-21.8
Communication (8.0)	14.5	3.4	14.6	3.4	8.0-20.0	14.4	3.4	7.0-20.0
RSB (5.0)	5.8	2.5	5.4	2.4	1.0-10.0	6.3	2.5	2.0-12.0

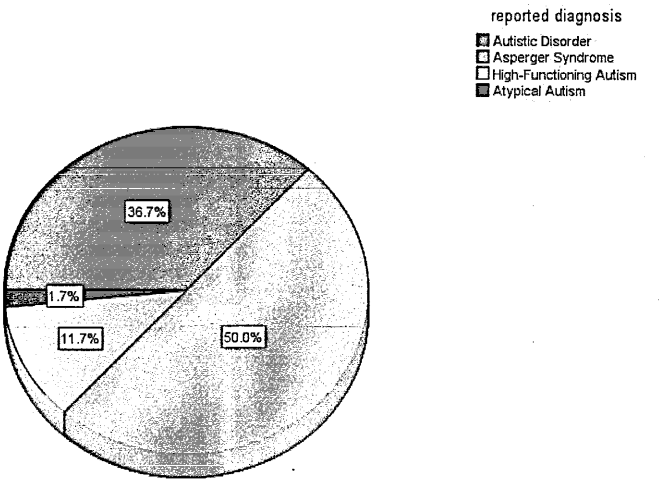
Table 3.9: *ADOS-G algorithm descriptives for the proband sample separated by module number.*[26]

ADOS Subscale	**All probands** (N = 56)								
	Module 2 (N = 5)			**Module 3** (N = 32)			**Module 4** (N = 19)		
	Median	Range	Clinical thresholds	Median	Range	Clinical thresholds	Median	Range	Clinical thresholds
Social interaction	8.0	1-13	3	8	3-14	4	7	2-14	4
Communication	7.0	2-9	4	2	0-8	2	4	0-7	2
S + C	15.0	5-22	8	10	4-21	7	11	4-21	7
RSB	0	0-1	n/a	0	0-4	n/a	0	0-4	n/a

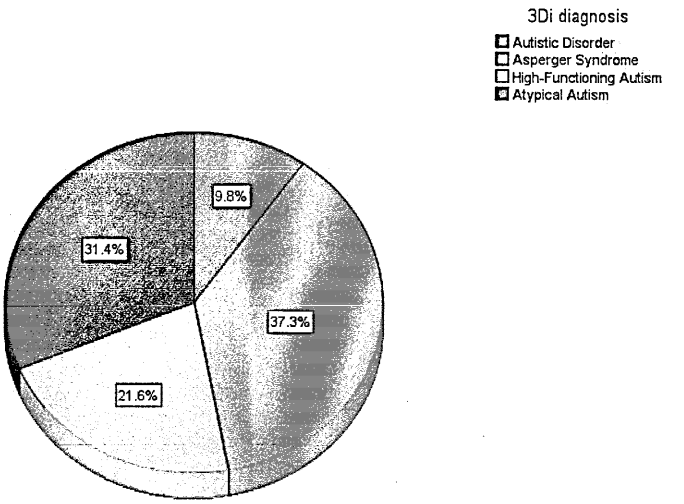
[24] PIQ: Performance IQ; VIQ: Verbal IQ. NB: A small number of probands failed to complete the IQ tests and so could not be given IQ scores.
[25] RSI: Reciprocal social interaction skills; RSB: Repetitive/ stereotyped behaviours and routines.
[26] S + C: Social interaction + communication total score; RSB: Repetitive, restrictive and stereotyped behaviour. NB: 6 probands failed to complete the assessment.

Figure 3.4: *Pie charts displaying (a) % of reported diagnostic sub-categories of ASC by parents (N = 60) and (b) % research sub-categories of ASC based on 3Di parental interview (N = 51).*[27] *Note: this figure is examining proband diagnostic category rather than proband ASC diagnosis verification. The data available is therefore different to that used for verifiying ASC diagnoses in section 3.6.*

(a)



(b)



[27] In (b) probands who met clinical criteria on all three subscales of the 3Di, had delayed language and received a non-verbal IQ score above 85 on the Raven's Progressive Matrices were given the label: High-Functioning Autism. NB: 2 families did not report the clinical diagnostic category of their child. Also, 6 probands did not meet clinical criteria for any diagnostic category on the 3Di and 5 probands could not be given a research diagnostic label because the mother could not remember the history of her child's language development, which is necessary to distinguish Asperger Syndrome from autistic disorder/ high-functioning autism.

Chapter Four

Using three self-report scales to explore the Broader Autism Phenotype in Multiplex versus Simplex Autism Families.

4.1 Abstract

Previous studies suggest that the BAP can be detected in the first-degree relatives of autistic probands using quantitative self-report scales of autistic traits and related phenotype. In this chapter, autistic traits and two related psychological constructs (empathy and systemising) were investigated in the unaffected parents of multiplex and simplex autism families and adult controls using three self-report measures: the Autism-Spectrum Quotient (AQ), Empathy Quotient (EQ) and Systemising Quotient-Revised (SQ-R). These measures were administered to 64 parents of multiplex families (32 mothers, 32 fathers), 60 parents of simplex families (30 mothers, 30 fathers) and 64 adult controls without any psychiatric conditions (32 females, 32 males). Contrary to the predictions, no significant differences were found between the three groups on each self-report scale. Significant sex differences were found for all three scales, with males self-reporting higher levels of autistic traits and related phenotypes than females. In contrast to previous studies, these findings using self-report measures do not provide evidence supporting the hypothesis that differential genetic mechanisms operate in simplex and multiplex autism families. Reasons for these discrepant findings are discussed.

4.2 Introduction

Research studies into the BAP reviewed in chapter one bolster the consensus view that genetic factors play a significant role in the biological aetiology of ASC and support the conceptualisation of ASC as a quantitative, dimensional and continuous phenotype that extends beyond people with an ASC diagnosis to include relatives of autistic probands and people in the general population (Baron-Cohen et al., 2001b; Constantino et al., 2006; Hoekstra et al., 2008). However, whilst the BAP is a well replicated finding, there are large inconsistencies in the research literature with some BAP characteristics receiving greater empirical support than others. It has therefore become important to understand and explain these mixed findings. One possible reason is that the samples of autistic probands and relatives used in studies on the BAP are too heterogeneous and need to be stratified. One opportunity for sample stratification receiving increasing interest comes from a recent hypothesis that BAP characteristics are largely restricted to the 'unaffected' relatives of multiple-incidence (multiplex) autism families, whilst the rate of BAP characteristics in the unaffected relatives of single-incidence (simplex) autism families is hypothesised to be significantly lower and similar to control groups (Constantino et al., 2010). These predicted differences can be inferred from a small number of autism genetic studies suggesting that there may be differential modes of genetic transmission operating in multiplex and simplex autism families (see chapter one, section 1.4.3). To restate these findings; de novo CNV have been implicated in ASC aetiology (e.g. Gauthier et al., 2009; Weiss et al., 2008), with some studies reporting higher percentages of de novo CNV in probands from simplex families compared to both

multiplex families and families without any history of psychiatric conditions (Marshall et al., 2008; Sebat et al., 2007). Furthermore, de novo Single Nucleotide Variants (SNV) have also been implicated in ASC aetiology; risk variants associated with previously identified ASC genes were found in probands that were not present in their unaffected parents or siblings (Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012). If de novo CNV and SNV play a major role in ASC aetiology and these risk variants are mainly found in simplex families, then this suggests that unaffected relatives from simplex families are less likely to contain a shared genetic vulnerability to ASC and therefore less likely to express ASC-related characteristics consistent with the BAP. Conversely, it has been hypothesised that unaffected relatives from multiplex families are more likely to share a number of common genetic risk variants of weak effect with the proband that collectively play a role in ASC aetiology and represent a shared genetic vulnerability to acquiring the condition (e.g. Pickles et al., 2000). If this is true then it is expected that relatives from families with more than one member with an ASC diagnosis are more likely to express characteristics consistent with the BAP.

In the disciplines of behavioural and cognitive psychology, sub-threshold autistic traits and related phenotypes have been investigated in the relatives of simplex and multiplex families using standardised interviews, observational assessments, informant-rated questionnaires and performance-based tasks. Interviews include the FHI (Bolton et al., 1994), which reviews ASC-related characteristics covering the DSM triad of impairments (communication, reciprocal social interaction and repetitive behaviours and interests) as well as personality characteristics and family history of other psychiatric conditions. A

study using the FHI by Szatmari et al. (2000) found significantly higher rates of social impairments in the biological relatives of multiplex families compared to simplex families but non-significant differences for communication impairments and repetitive activities. More recently, measures have been devised that take into account the quantitative nature of the autism phenotype. These include an interview and observational assessment called the Broader Phenotype Autism Symptom Scales (BPASS; Dawson et al., 2007). A study by Bernier et al. (2012) used the BPASS to assess BAP characteristics in multiplex versus simplex relatives and a clinical/ non-clinical control group. Significantly higher levels of BAP characteristics were present in multiplex parents compared to simplex parents and the two control groups for one domain (social motivation/ interest) and significantly higher than simplex parents only for the 'conversational skills' domain.

Although interviews and observational assessments provide important insights into the BAP, they both suffer from a number of limitations. For example, interviews are often time-consuming and stressful for participants whilst the capacity for researchers to accurately rate answers is constrained by the quality of the participant's verbal response. Observational assessments, on the other hand, are constrained by a small period of time in which to observe ASC-related characteristics in the participant and observations are restricted to specific circumstances and contexts. Furthermore, it is not clear how naturalistic the observational assessments of social behaviour in ASC parents are in BAP research studies, where the research setting is likely to increase anxiety in some participants. Furthermore, psychogenic factors may be a greater problem in observational

assessments such as a reduced confidence in one's own social skills as a result of having a prior awareness of the familial nature of ASC. Finally, researchers who rate the observational assessments may be susceptible to coding biases if the participant provides information that give clues indicating whether she/he has family members with an ASC diagnosis.

Other studies have examined differences in BAP expression among simplex and multiplex relatives using informant-rated questionnaires. These include a study by Constantino et al. (2010) using the SRS; a questionnaire completed by parents that quantitatively assesses autistic traits and symptoms. Parents from multiplex and simplex autism families completed the SRS about their unaffected children. Results revealed an aggregation of quantitative autistic traits in the unaffected siblings of multiplex families, especially male siblings which were significantly higher than simplex male siblings, whilst high levels of autistic traits were largely absent in the unaffected siblings of simplex families. However, these results were not replicated in a study of multiplex and simplex relatives using the SRS by De la Marche et al. (2012).

In this current investigation the BAP was investigated in multiplex and simplex autism relatives and controls using three *self*-rated questionnaires; the AQ, EQ and SQ-R (Baron-Cohen et al., 2001b; Baron-Cohen and Wheelwright, 2004; Wheelwright et al., 2006; see chapter three). Self-rated questionnaires hold advantages over informant-rated questionnaires e.g. a person rating themselves can reflect upon and assess their level of social functioning in a wide range of contexts and environments whilst informants are

normally restricted to observing the rated person in specific environments (e.g. the home or workplace). On the other hand, self-rated reports are less objective measures and people's perceptions of their own social functioning may not always be an accurate reflection of their true capabilities or deficits, especially if they have poor social and emotional insights- the very traits explored by some of these measures. For this reason, self-report questionnaires also assess people's habits and preferences as well as their abilities. Both self and informant-rated questionnaires, however, hold an advantage over interviews and observational assessments by allowing people to complete the measure(s) in their own time, which places less stress and demands upon participants.

A number of previous studies have provided evidence for the BAP using the AQ as a self-report questionnaire e.g. in an online study by Wheelwright et al. (2010), 571 fathers and 1429 mothers of children with ASC reported significantly higher total AQ scores than the parents of typically developing children as well as 4 out of the 5 theoretical subscales originally suggested in a study by Baron-Cohen et al. (2001b). Likewise, a study by Ruta et al. (2011) using a clinical sample in Italy reported significantly higher total AQ scores in the parents of children with ASC compared to parents of typically developing children, as well as for two subscales (social skills and communication). These findings replicated an earlier study by Bishop et al. (2004) that found significantly higher AQ scores in ASC relatives versus controls on the same two subscales. However, none of these studies stratified ASC relatives into multiplex and simplex categories and explored differences in the expression of the BAP in these two groups.

This investigation is the first to examine differences in BAP characteristics in multiplex versus simplex autism parents and controls using the AQ. It is also the first to investigate the BAP in the relatives of multiplex versus simplex autism families using self- (rather than informant-) rated questionnaires of autistic traits and related cognitive phenotype. The EQ and SQ-R have not been previously published in studies on the BAP. However, cognitive characteristics associated with empathy and systemising have been investigated, such as facial emotion recognition, complex mental state recognition (associated with empathy; see chapter one, section 1.3.2.1) and visual-spatial/ attention to detail ability (associated with systemising; see chapter one, section 1.3.2.3). Many of these characteristics have been reported as significantly different in the relatives of autistic probands compared to controls, but not all results have been consistent (Baron-Cohen and Hammer, 1997; Bölte and Poustka, 2003; Losh et al., 2009; Scheeren and Stauder, 2008; Wallace et al., 2010). With regards to the EQ, results from chapter two suggested that fathers, but not mothers, of children with ASC reported significantly lower (i.e. more impaired) EQ scores than adults from the general population. The study here aimed to assess whether significantly poorer empathy and significantly stronger systemising was self-reported in multiplex parents compared to simplex parents and controls from the general population. In doing so, this would provide support that these constructs are relevant in the operational characterisation of the BAP and offers evidence consistent with the hypothesis that different genetic mechanisms operate in simplex and multiplex autism families. Furthermore, this study aimed to assess whether the AQ, as a more general measure of autistic traits, would detect differences between multiplex

parents, simplex parents and controls providing further support for this genetic hypothesis (see chapter one, section 1.4.3).

4.3 Predictions

Predictions for this investigation were three-fold: (1) Multiplex autism parents will self-report higher levels of quantitative autistic traits on the AQ (especially on the higher order 'social interaction' factor subscale; see section 4.4.3) than simplex autism parents who in turn will self-report higher or equal levels of quantitative autistic traits on the AQ than controls from the general population (multiplex > simplex ≥ control), (2) Multiplex autism parents will self-report significantly lower empathy on the EQ than simplex autism parents who in turn will self-report lower or an equal level of empathy on the EQ than controls from the general population (Multiplex < Simplex ≤ Control), (3) Multiplex autism parents will self-report significantly higher systemising on the SQ-R than simplex autism parents who in turn will self-report higher or an equal level of systemising on the SQ-R compared to controls (Multiplex > Simplex ≥ Control). In addition to these analyses, correlations were examined between scores on the three self-report questionnaires and education level/ standardised scores on the Raven's Progressive Matrices (a short measure of non-verbal IQ). It was expected that the scores on these self-report questionnaires would be largely independent of education and non-verbal intelligence.

4.4 Methods

4.4.1 Participants

For information about participant recruitment see chapter three, section 3.3. After applying proband verification criteria and simplex/multiplex classification criteria (see sections 3.6 and 3.7), total sample sizes were as follows: 60 simplex parents (30 mothers, 30 fathers) and 64 multiplex parents (32 mothers, 32 fathers). Parents could choose to complete the AQ, EQ and SQ-R offline or online via the Cambridge University Autism Research Centre website (see Table 4.1). Participants completed the Raven's Progressive Matrices (SPM+ version) and the Mill-Hill vocabulary Scale on the testing day. The simplex and multiplex parent groups did not significantly differ on non-verbal IQ (using the Raven's Progressive Matrices; $p = 0.74$) and verbal IQ (using the Mill-Hill vocabulary scale; $p = 0.40$).

The control group was taken from the same sample of participants used in chapter two; participants were recruited online via the Cambridge University psychology database (see chapter two, section 2.3.1). All participants below the age of 34 years were removed so that this group did not significantly differ from the simplex and multiplex parent groups on age ($p = 0.61$). Control participants had completed an online adaptation of the Raven's Progressive Matrices. However, this was not comparable to the offline version completed by the ASC parent groups and so it was not possible to match the control group on non-verbal IQ. The control group had provided information about educational qualifications

and so it was possible to match the control group to the ASC parent groups on educational level; the percentage of people in each group with a higher educational qualification were not significantly different from each other ($p > 0.05$; see Table 4.1). The total number of control participants available for data analysis was 64 (32 males, 32 females).

Table 4.1: *Summary of mean (SD) ages and IQ, plus education level and test administration formats for each group.*[28]

	Multiplex Parents	**Simplex Parents**	**Controls**
N	64	60	64
Mean Age (SD)	44.5 (5.3) [1]	44.9 (7.3)	43.8 (8.3)
Non-verbal IQ (SD)	96.9 (11.4) [2]	97.6 (11.1)	-
Verbal IQ (SD)	98.3 (13.9) [2]	100.4 (13.4)	-
% with higher education qualification	52.5 [5]	60.7 [4]	60.9
% completing AQ offline	93.7 [1]	70 [2]	0
% completing AQ online	6.3 [1]	30 [2]	100
% completing EQ offline	93.7 [1]	71.9 [3]	0
% completing EQ online	6.3 [1]	28.1 [3]	100
% completing SQ-R offline	93.8	62.1 [2]	0 [2]
% completing SQ-R online	6.3	37.9 [2]	100 [2]

4.4.2 Materials and procedure

Participants completed three self-report questionnaires (the AQ, EQ and SQ-R). The majority of simplex and multiplex parents completed these questionnaires offline (pencil and paper versions) whilst all control adults completed them online (see Table 4.1). Furthermore, ASC parents completed the Raven's Progressive Matrices (SPM+ version)

[28] Numbers in superscript indicate total amount of people within sample that failed to complete measure or to provide appropriate information in each sample.

and the Raven's Mill-Hill Vocabulary Scale to measure non-verbal and verbal IQ respectively. For further details about these measures, see chapter three (sections 3.5.2-3.5.4 and 3.5.9-3.5.10). Proband diagnoses were verified using the 3Di-short and the ADOS-G (see sections 3.5.11-3.5.12 and 3.6 for further details).

4.4.3 Statistical Analyses

AQ, EQ and SQ-R scores were firstly analysed by conducting two-way ANOVAs with sex and group (multiplex parents, simplex parents and adult controls) as the two between-subject factors.

For the AQ, both total score and two subscale scores were selected as the dependent variables (a higher order 'social interaction' factor and an 'attention to detail' factor). These subscales were selected because they are the outcome of an extensive factor analysis of the AQ using a large sample size comprising students and participants from the general population (Hoekstra et al., 2008). Therefore, the separation of the AQ into a higher order social interaction factor and a non-social/ attention to detail factor appears to be empirically meaningful. For the EQ, total score was selected as the dependent variable because the most extensive analysis of the factor structure of this self-report measure suggests a single dimension (Allison et al., 2011) and so it is therefore considered acceptable to use a single summed total score, rather than previously suggested subscales (e.g. Lawrence et al., 2004). Finally, total SQ-R score was used as a dependent variable in line with previous studies on systemising using this measure (e.g. Baron-Cohen et al.,

2003; Wheelwright et al., 2006). An extensive factor analysis has yet to be carried out on the revised version of this measure. However, a previous study of the SQ-R has indicated good internal consistency (Cronbach's alpha = 0.90; Wheelwright et al., 2006), which suggests that it is acceptable to use the sum score for this questionnaire.

4.5. Results

Table 4.2: *Descriptives for the AQ (including factor subscales), EQ and SQ-R.*

Group	Sex		AQ (total)	AQ (Social Interaction factor)	AQ (Attention to Detail)	EQ (total)	SQ-R (total)
Multiplex Parents	Male	**N**	31	31	31	31	32
		Mean	118.5	94.5	24.0	33.4	61.9
		SD	20.7	17.7	5.6	14.8	16.1
	Female	**N**	32	32	32	32	32
		Mean	101.8	79.8	21.9	50.3	44.7
		SD	28.9	25.0	6.8	15.8	21.1
	Total	**N**	63	63	63	63	64
		Mean	110.0	87.0	23.0	42.0	53.3
		SD	26.4	22.8	6.3	17.4	20.6
Simplex Parents	Male	**N**	28	28	28	28	28
		Mean	115.4	90.4	25.0	36.8	66.6
		SD	19.8	18.3	4.2	11.9	21.6
	Female	**N**	30	30	30	29	30
		Mean	100.3	78.5	21.8	53.0	44.5
		SD	22.4	19.4	5.0	14.3	16.7
	Total	**N**	58	58	58	57	58
		Mean	107.6	84.3	23.4	45.0	55.2
		SD	22.4	19.7	4.9	15.4	22.1
Control	Male	**N**	32	32	32	32	30
		Mean	117.0	91.2	25.6	37.7	67.5
		SD	18.5	16.0	5.9	14.8	28.2
	Female	**N**	32	32	32	32	32
		Mean	99.9	76.1	23.8	49.6	56.8
		SD	18.6	18.2	4.4	14.6	21.6
	Total	**N**	64	64	64	64	62
		Mean	108.7	83.6	24.7	43.6	61.9
		SD	23.1	18.6	5.2	15.8	25.4

4.5.1 Self-rated autistic traits (AQ)

Mean AQ scores, standard deviations and sample sizes in each group are displayed in Table 4.2. A group × sex Analysis of Variance (ANOVA) was carried out on total score and the two subscale factors identified using a confirmatory factor analysis by Hoekstra et al. (2008).

When total score was the dependent variable, results of the 2-way ANOVA indicated a significant main effect of sex ($F(1,179) = 25.53$, $p < 0.001$, $r = 0.22$). There was a non-significant main effect of group ($p > 0.05$) and a non-significant interaction between group and sex ($p > 0.05$).

On the 'social interaction' factor subscale, results indicated there was a significant main effect of sex ($F(1,179) = 23.84$, $p < 0.001$, $r = 0.22$) whilst both group and the group × sex interaction were non-significant ($p > 0.05$).

Finally, on the 'Attention to detail' factor subscale, results of the 2-way ANOVA revealed a significant main effect of sex ($F(1,179) = 8.69$, $p < 0.01$, $r = 0.10$). Both the main effect of group and the group × sex interaction were non-significant ($p > 0.05$).

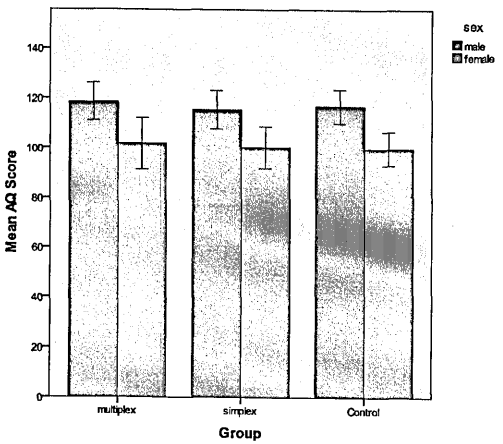
In summary, the main effect of group was non-significant for all three AQ scales. A graphical representation of these results is provided in Figure 4.1.

4.5.2 Self-rated empathy (EQ)

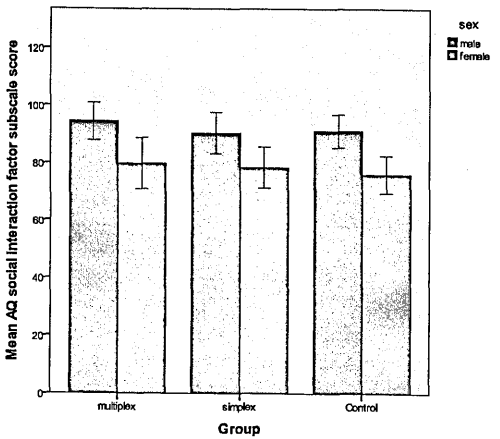
Mean EQ scores, standard deviations and sample sizes for each group are displayed in Table 4.2. The mean EQ score for male controls (37.7) was the same as the equivalent mean from the study reported in chapter two that used a larger sample of male controls, whilst the mean EQ score for female controls (49.6) was slightly above the equivalent mean from the study reported in chapter two (48.5), which used a larger sample of female controls. A group × sex analysis of variance (ANOVA) was carried out on mean EQ score. Results of the 2-way ANOVA revealed a significant main effect of sex ($F(1,178) = 49.72$, $p < 0.001$, $r = 0.24$). Conversely, the main effect of group was non-significant ($p > 0.05$) and the group × sex interaction was also non-significant ($p > 0.05$). For a graphical representation of the results, see Figure 4.2.

Figure 4.1: *Bar graphs displaying the main effects of group and sex on (a) Total AQ scores, (b) Social Interaction factor subscale scores and (c) Attention to detail factor subscale scores.*[29]

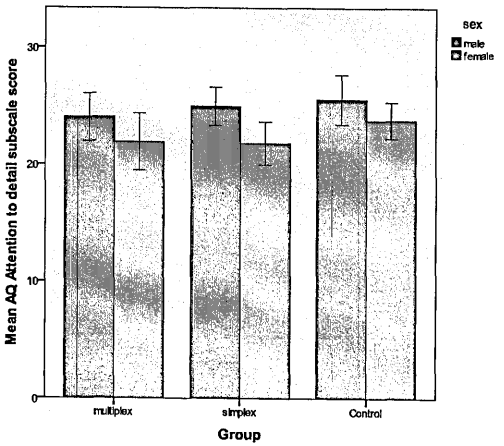
(a)



(b)

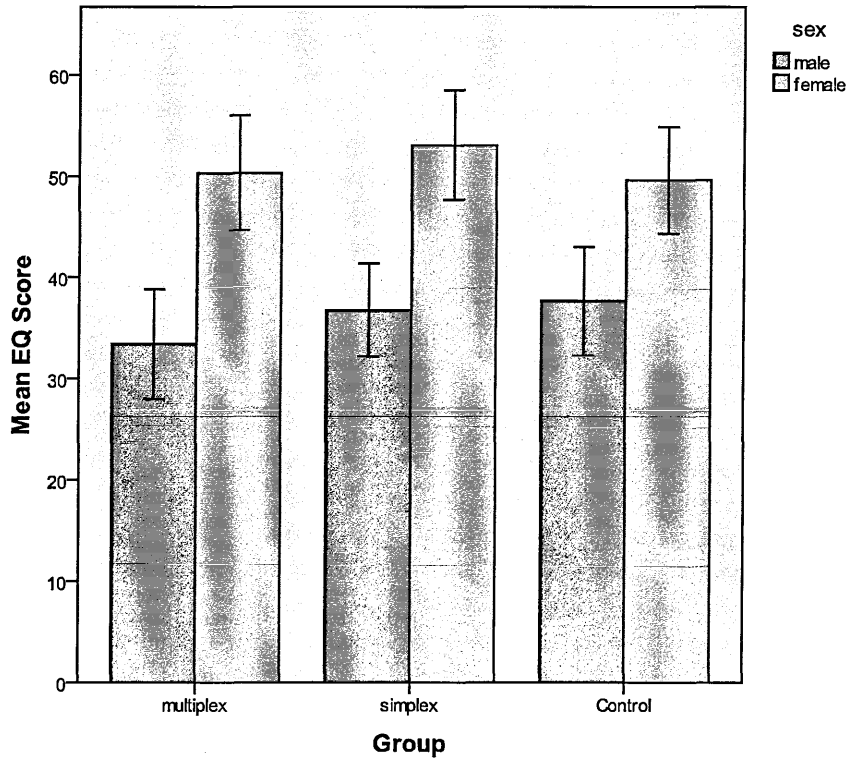


(c)



[29] AQ: Autism spectrum Quotient; Error bars represent 95% confidence intervals.

Figure 4.2: *Bar graph displaying the main effects of group and sex on mean EQ score.*[30]



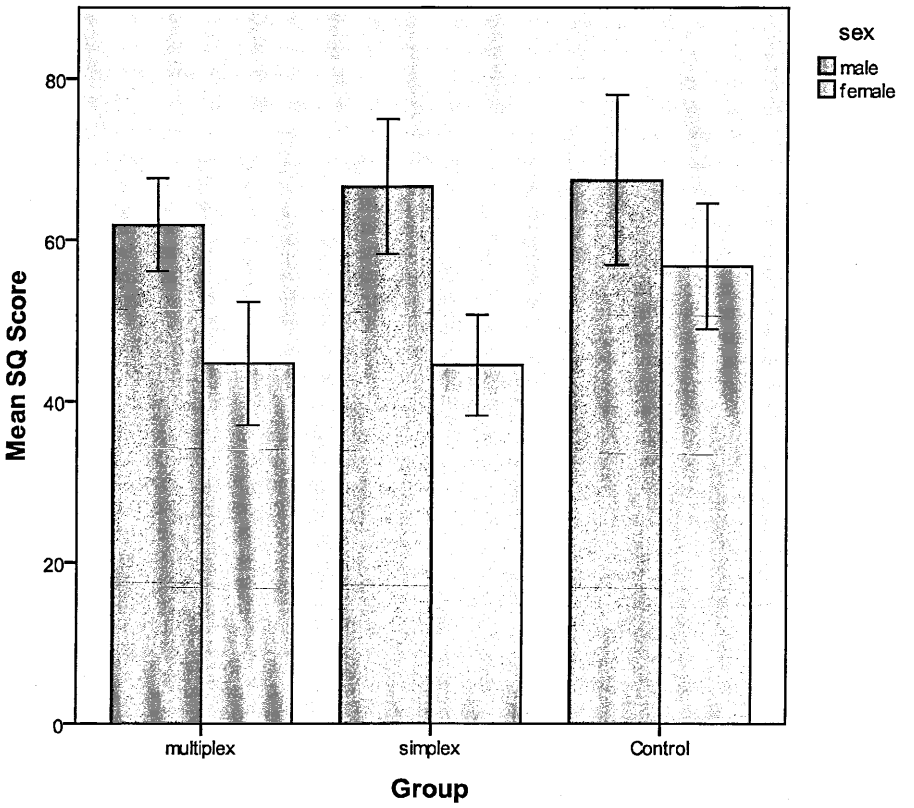
4.5.3 Self-rated systemising (SQ-R)

See Table 4.2 for mean SQ-R scores, standard deviations and sample sizes for each group. Mean SQ-R scores for male and female controls (67.5 and 56.8 respectively) were much higher than the sex-equivalent means from an original study using the SQ-R (61.2 and 51.7 respectively; Wheelwright et al. 2006). A group × sex analysis of variance (ANOVA) was carried out on mean SQ-R score. Results of the 2-way ANOVA revealed a significant main effect of sex ($F(1,178) = 28.42$, $p < 0.001$, $r = 0.15$). However the main

[30] EQ: Empathy Quotient; Error bars represent 95% confidence intervals.

effect of group and the group × sex interaction were both non-significant ($p > 0.05$) (see Figure 4.3).

Figure 4.3: *Bar graph displaying the main effects of group and sex on mean SQ-R score.*[31]



4.5.4 Correlations with non-verbal IQ and education level

To discern whether non-verbal IQ significantly co-varied with any of these self-report measures, correlation coefficients were obtained. Amongst the parents of autistic probands there was a non-significant relationship between AQ score and standard score on the Raven's Progressive Matrices (Pearson correlation; $p > .05$) and a non-significant

[31] SQ-R: Systemising Quotient-Revised; Error bars represent 95% confidence intervals.

relationship between EQ score and standard score on the Raven's Progressive Matrices (Pearson correlation; $p > 0.05$). However, there was a significant positive relationship between SQ-R score and standard score on the Raven's Progressive Matrices (Pearson correlation $r = .31$, p (two-tailed) < 0.01).

Spearman correlations were carried out between AQ, EQ and SQ-R scores and participants' highest educational qualification. Control participants had provided information on whether they had a completed a higher education qualification (see Table 4.1), but no further information about education level was recorded; only ASC parents had given extensive enough information about their highest education qualification to make it possible to carry out correlations with the self-report scales. Amongst parents of autistic probands, there were non-significant relationships between total AQ/ AQ 'social interaction'/ AQ 'attention to detail' subscale scores and highest educational qualification (all p (two-tailed) > 0.05). There was also a non-significant relationship between total EQ scores and highest educational qualification (p (two-tailed) > 0.05) but a significant positive relationship between total SQ-R scores and highest educational qualification ($\rho = .19$, p (two-tailed) < 0.05).

4.6 Discussion

This investigation is the first to use self-report scales (the AQ, EQ and SQ-R) to examine differences in BAP characteristics in the unaffected first-degree relatives (parents) of multiplex versus simplex autism families. If multiplex parents display a significantly

higher aggregation of autistic traits and related phenotypes compared to simplex parents and controls then this is consistent with the hypothesis that different genetic mechanisms are operating in multiplex and simplex autism (Sebat et al., 2007; Abrahams and Geschwind, 2008). Results did not confirm this prediction; mean scores on the questionnaires were broadly similar across the three groups. There was no evidence for sex-specific expression of the BAP, although there were significant sex differences on all questionnaires across the three groups, with males scoring significantly higher autistic traits and self-reporting superior systemising and poorer empathising than females.

The failure to detect differences between multiplex parents, simplex parents and controls on the AQ is particularly surprising, given that a number of previous studies have detected differences between ASC parents and controls (e.g. Bishop et al., 2004; Ruta et al., 2011; Wheelwright et al., 2010). Here it was expected that multiplex parents, who have more than one child with an ASC diagnosis, would self-report significantly higher AQ scores than controls and simplex parents, but this was not found. These negative results suggest that the AQ may not be sufficiently sensitive at detecting subtle differences between simplex and multiplex relatives, nor between ASC parents and controls, at least in relatively modest sample sizes, as reported here.

With regards to the EQ, results in chapter two indicated that ASC fathers self-reported significantly lower EQ scores than adult males. However, this result was not replicated here using a new sample of participants although mean EQ scores for multiplex ASC fathers and male controls were similar (33.4 and 38.1 respectively compared to 32.2. for

ASC fathers and 37.7 for male controls in chapter two). Perhaps if power was increased by using larger sample sizes then a significant difference would have been detected between multiplex parents and controls on the EQ. Finally, with regard to the SQ-R, these results did not find significant differences in the predicted direction for self-reported systemising across the three groups, which suggests that this construct is not a reliable marker of the BAP or a feature of the underlying genetic vulnerability to ASC.

Mean score on the SQ-R and the AQ attention to detail subscale in controls was higher than simplex and multiplex parent samples, although not statistically significant; this was especially true of the SQ-R in female controls compared to simplex and multiplex mothers. In a study on the BAP by Scheeren and Stauder (2008), female controls scored significantly higher than mothers of autistic probands on the AQ attention to detail subscale. Scheeren and Stauder suggested that controls may have perceived the 'attention to detail' items of the AQ as positive, causing them to provide socially desirable answers (i.e. high attention to detail), whilst parents of ASC children may have recognised these items as features of ASC and so perceived them as negative attributes. This may have made ASC parents more reluctant to report themselves as having high attention to detail leading to the significant differences found in their study. Perhaps a similar phenomenon accounts for the lack of predicted differences between groups on the non-social scales in this current study.

It is also important to consider why there were no significant differences (in the predicted directions) in the aggregation of autistic traits and related phenotype in the *social* domain

between multiplex parents, simplex parents and controls. Firstly, it is possible that parents of children with ASC, who are more likely to be aware of ASC symptoms than controls, did not want to self-report high levels of autistic traits. Rather, they may have given socially desirable answers instead of responses that accurately reflected their true beliefs and abilities. This may have been most pronounced in parents of families where more than one child has an ASC diagnosis (i.e. multiplex families). Secondly, it is possible that the ASC parents in these samples do display milder ASC-related characteristics but these subtle differences have been sufficiently compensated for in their day-to-day lives and so were not detected using self-report questionnaires such as the AQ and require more objective measures that aim to assess specific features of ASC symptomatology (e.g. performance-based social cognition tests; see chapter five). However, given previous reports of significant differences between ASC parents and controls using self-report measures (including the AQ) this seems unlikely. Further studies are needed using similar self-report scales of autistic traits and related phenotypes to discern whether the same pattern of results is independently found in new samples of multiplex and simplex relatives.

There were a small number of limitations to acknowledge in this study. Firstly, the control group would have been a more suitable comparison group if it consisted of parents of typically developing children. The control group used here may have contained a large number of single adults without families, which could have had an influence on how these self-report questionnaires were answered (e.g. participants without families may have greater freedom and time to pursue skills and interests associated with

systemising). Furthermore, it would have been useful to compare multiplex and simplex parents to a clinical control group, such as the parents of children with Down Syndrome (e.g. see Losh et al., 2008). Secondly, the control group could not be matched for non-verbal and verbal IQ. Even though groups were matched for educational level, the lack of significant differences between multiplex/ simplex parents and controls may be attributed to possible hidden differences in general cognitive functioning; this may be especially true for the SQ-R which was found to significantly correlate with non-verbal IQ (see section 4.5.4). Thus it is important to control for IQ in future studies of the SQ-R in ASC parents.

Thirdly, due to time restrictions the questionnaires used in this investigation were self-report format only and so it would have been an improvement to use both self and informant report questionnaires. This helps protect against inaccuracies caused by participants who provide socially desirable responses to items rather than their true beliefs and capabilities.

Despite these limitations, this investigation has a large number of strengths. It is the first to investigate BAP characteristics in simplex and multiplex autism families using self-report scales. Secondly, it uses more comprehensive and stringent criteria than previous studies for assigning simplex and multiplex status to families (e.g. Virkud et al., 2009; see chapter three, section 3.7). Thirdly, one proband diagnosis from each family could be verified using the ADOS-G and 3Di-short (see chapter three, section 3.6). Fourthly,

multiplex and simplex parents could be accurately matched on verbal IQ, non-verbal IQ and age.

In summary, these self-report measures do not provide support for greater aggregation of self-rated autistic traits and related phenotypes (lower self-rated empathy/ higher self-rated systemising) in the parents of multiplex families compared to simplex parents and controls, and by extension these results are not consistent with the hypothesis of differential genetic mechanisms operating in multiplex and simplex autism. Furthermore, neither simplex parents nor multiplex parents self-reported a significantly higher level of quantitative autistic traits and related phenotypes compared to controls. Therefore, this study of the BAP using self-report measures did not generate the differences predicted on the basis of earlier work (Bishop et al., 2004; Ruta et al., 2011; Wheelwright et al., 2010). The next chapter extends these studies by focusing more on the 'cognitive' level of analysis, discerning whether the predicted pattern of results can be found for three performance-based tasks, which span both the social and non-social domains of ASC.

Chapter Five

Using three performance-based tasks to explore the Broader Autism Phenotype in Multiplex versus Simplex Autism Families.

5.1 Abstract

Previous studies suggest that the unaffected first-degree relatives of people with ASC display mild difficulties or superiorities on neuropsychological tasks compared to control groups, reflecting a milder expression of the full clinical phenotype at a cognitive level. Some of these studies have suggested that this broader cognitive phenotype is restricted to a subset of genetic relatives, but only one has assessed whether it is restricted to the relatives of multiplex autism families. Here, for the first time, the parents of multiplex and simplex autism families were administered a battery of neuropsychological tasks spanning the social and non-social domains of ASC. These included the KDEF and Mind in Eyes tasks assessing emotion and complex mental state perception, and a visuospatial task assessing attention to detail (the EFT). Results suggest that multiplex parents tend to have significantly poorer mentalizing ability than simplex parents; they were significantly less accurate at identifying complex mental states from the eye region of the face, after controlling for verbal intelligence ($p < 0.05$). Furthermore, when KDEF accuracy-adjusted response time was used as the dependent variable, results suggested that multiplex mothers were significantly poorer than simplex mothers at recognising fear from facial expressions ($p < 0.01$), whilst there was no significant difference between multiplex fathers and simplex fathers. Furthermore, using the same dependent variable, multiplex fathers, but not multiplex mothers, were significantly poorer on average than sex-matched controls at identifying sad facial expressions ($p < 0.05$). Thus, results overall suggest that the parents of multiplex autism families may be significantly poorer at recognising specific, negative basic emotions, which here includes sadness and fear.

There were no significant differences in performance between simplex parents and controls on the emotion/ mental state perception tasks and all significant differences reported were between multiplex parents and simplex parents or controls. On the visuospatial task, no significant differences were found across the groups. These results provide some support for the hypothesis that differential genetic mechanisms operate in simplex and multiplex autism, but in the social domain only. Social cognitive difficulties, implicated by significantly lower scores on tests of emotion/ mental state perception, may represent an underlying genetic liability for ASC that aggregates in the first-degree relatives of probands from multiplex autism families.

5.2 Introduction

Performance-based cognitive tasks offer important insights into whether people with clinical ASC receive and process information differently from people without a clinical diagnosis. These tasks have varied widely, assessing various domains of functioning, including (1) social cognition, associated with the social and communication impairments of ASC (Ashwin et al., 2006; Baron-Cohen et al., 2001a; Happé, 1994) and (2) sensory attention and perception, associated with the non-social, restricted repetitive behaviours of ASC (Baron-Cohen et al., 2009; Frith and Happé, 1994; Jolliffe and Baron-Cohen, 1997, Pellicano et al., 2005). Whilst studies have not always been consistent, many have reported impairments or superiorities in these domains in people with clinical ASC. In addition to these findings, the same or similar performance tasks have been administered to first-degree relatives of people with ASC to assess whether deficits or superiorities in various perceptual/ cognitive domains are associated with the BAP; investigating autistic traits and related phenotypes in first-degree relatives could help to identify the heritable features of the ASC phenotype (see chapter one for a comprehensive overview).

Whilst the research literature on the BAP at a 'cognitive' level is somewhat inconsistent, areas that have received some of the greatest support include attenuated performance on tasks involving social cognition and emotion perception. These include differences between ASC parents/ siblings and controls on tests of basic emotion recognition (Losh et al., 2009; Palermo et al., 2006; Wallace et al., 2010), face processing strategy (Adolphs et al., 2008), complex mental state recognition (Baron-Cohen and Hammer, 1997, Dorris

et al., 2004; Losh and Piven, 2007; Losh et al., 2009), emotional judgement/ mental state reasoning (Gokcen et al., 2009; Losh et al., 2009) and facial identity recognition (Wilson et al., 2010).

Furthermore, there is modest evidence suggesting that first-degree relatives demonstrate superior performance on visuospatial tasks assessing attention to detail, including the EFT (Baron-Cohen and Hammer, 1997; Bölte and Poustka, 2006; Happé et al., 2001) and the BDT (Happé et al., 2001). Happé et al. (2001) reported significant differences in male relatives (fathers) only. These findings echo reports of superiorities in the same cognitive domains in people with clinical ASC (e.g. Jolliffe and Baron-Cohen, 1997; but see White and Saldaña, 2011).

This study used performance-based tasks in both the social and non-social domains to explore possible differences between the parents of multiplex autism families, simplex autism families and age- and education-matched controls. All previous studies investigating the cognitive profile of the BAP have examined differences between ASC relatives and controls only (e.g. Baron-Cohen and Hammer, 1997; Belmonte et al., 2010) or between relatives who have been stratified into groups according to personality characteristics associated with the BAP ('BAP+' versus 'BAP-' and controls; Losh et al., 2009). This is one of the first studies to explore the cognitive profile of the BAP by stratifying ASC parents into multiplex and simplex groups (see chapter three, section 3.7 for simplex/ multiplex classification criteria). Exploring the cognitive profile of the BAP using these stratified samples can be considered an improvement upon studies

investigating the BAP in unstratified ASC relatives and controls. By focusing on differences between multiplex and simplex relatives and controls, one is testing a more specific hypothesis derived from a number of autism genetic studies (see chapter one, section 1.4.3). BAP characteristics in multiplex relatives may thus represent an underlying genetic vulnerability that can be detected using cognitive tasks, whilst simplex parents may not share a similar vulnerability. Some studies have found significant differences on performance-based tasks in parents stratified into 'BAP+' and 'BAP-' groups (e.g. Losh and Piven, 2007; Losh et al., 2009). However, these findings are somewhat expected because parents have been classified prior to analyses according to personality features that are associated with ASC symptomatology (e.g. Losh and Piven (2007) classified parents into BAP+ or BAP- groups depending on whether they reported an aloof personality, which describes people who are disinterested in social interaction). In contrast, this study takes a different approach by examining average differences between groups based on their expected genetic vulnerability rather than their behavioural profile.

Previous studies have examined differences between the unaffected relatives of multiplex and simplex autism families using questionnaires, interviews or observational assessments (see chapter four), but only one previous study used a performance-based task (Bölte and Poustka, 2003). This current study uses two social cognition tasks that assess people's ability to recognise simple and complex emotions/ mental states and a non-social performance-based task that assesses people's attention to detail. The former tests were chosen because a literature review on the BAP indicates that some of the

strongest support for a BAP is found for tests of social cognition/ emotion perception (see chapter one), whilst the latter test was chosen because it is conceptually associated with the non-social behavioural features of clinical ASC and takes into account the cognitive strengths that are thought to be part of the ASC phenotype rather than focusing exclusively on impairments in perception/ cognition.

5.3 Predictions

The predictions for this study are three-fold and are based on the behaviour genetic hypothesis that unaffected members of multiplex families are more likely to possess inherited genetic risk variants for ASC of weak effect that give rise to the BAP, whilst this is less likely for the unaffected members of simplex families where the genetic risk variants are hypothesised to more often arise de novo and so by definition are not shared by other family members (see chapter one, section 1.4.3 for a summary of the relevant findings from autism genetic studies). The three predictions are as follows: (1) Multiplex autism parents would perform significantly worse than simplex autism parents and controls on a test of complex mental state recognition. Simplex autism parents would perform similarly to controls, or display a mild impairment that is less severe than multiplex parents. This prediction can be summarised as: Multiplex parents < Simplex parents ≤ Controls. (2) Multiplex autism parents would perform significantly worse than simplex autism parents and controls on a test of basic facial emotion recognition, particularly when the dependent variable is accuracy-adjusted response time. Furthermore, it is expected that simplex autism parents will either perform similarly to

controls or show a mild impairment that is less severe than multiplex parents. These predictions can be summarised as: Multiplex parents < Simplex parents ≤ Controls. Specifically, it is expected that multiplex parents will be significantly worse, on average, at recognising basic negative emotions (sad, angry, afraid and disgust) as has been reported in previous research studies in unstratified samples of ASC relatives or ASC relatives stratified according to their behavioural profile (Losh et al., 2009; Palermo et al., 2006; Wallace et al., 2010; but see chapter two). (3) Multiplex autism parents would perform significantly better than simplex autism parents on the EFT, reflected by significantly higher accuracy scores and significantly lower response times. Control data for this task was not obtained so only multiplex and simplex parents could be compared. These predictions can be summarised as: Accuracy: Multiplex parents > Simplex parents, Response Time: Multiplex parents < Simplex parents.

5.4 Methods

5.4.1 Participants

After applying simplex/ multiplex classification criteria and proband diagnosis verification criteria (see chapter three; sections 3.6 and 3.7), the number of ASC parents available for analyses were as follows: 64 multiplex parents (32 mothers, 32 fathers) and 60 simplex parents (30 mothers, 30 fathers). Parents could choose to complete the social cognition tasks offline on a laptop during the testing day or complete the same computer versions of the tasks online prior to the testing day via the Cambridge University Autism

Research Centre website (see Table 5.1 for percentages). All participants completed the visuospatial task (EFT) during the testing day, as well as the Raven's Progressive Matrices SPM+ version (a measure of non-verbal IQ) and the Mill-Hill Vocabulary Scale (a measure of verbal IQ). See chapter three for a full description of these measures. The multiplex and simplex parent groups did not significantly differ on both non-verbal IQ ($p = 0.74$) and verbal IQ ($p = 0.40$). A small minority of ASC parents failed to complete specific measures and so there was some missing data on each test; the final sample sizes are displayed in Table 5.1.

The control group was taken from the same sample of participants used in chapters two and four; they were adults without any psychiatric conditions and without a family history of ASC. They were recruited online via the Cambridge University psychology database (www.cambridgepsychology.com). Multiplex parents, simplex parents and control participants were matched on age ($p = 0.69$) and education level ($p = 0.57$). These control participants had not completed a comparable measure of verbal/ non-verbal IQ and so the group could not be matched for IQ. Control participants had also not completed the EFT and so the data from multiplex and simplex parents could not be compared to data from controls for this measure.

Table 5.1: *Summary of mean (SD) ages and IQ, plus education level and test administration formats for each group.*[32]

	Multiplex Parents	Simplex Parents	Controls
N	64	60	64
Mean Age (years) (SD)	44.5 (5.3) [1]	44.9 (7.3)	43.8 (8.3)
Non-verbal IQ (SD)	96.9 (11.4) [2]	97.6 (11.1)	-
Verbal IQ (SD)	98.3 (13.9) [2]	100.4 (13.4)	-
% with higher education qualification	52.5 [5]	60.7 [4]	60.9
% completing Mind in Eyes offline	96.8 [2]	69.1 [5]	-
% completing Mind in Eyes online	3.2 [2]	30.9 [5]	-
% completing KDEF offline	96.8 [4]	78.3 [2]	0
% completing KDEF online	3.2 [4]	21.7 [2]	100
% completing EFT	96.9 [2]	100	-

5.4.2 Materials and procedure

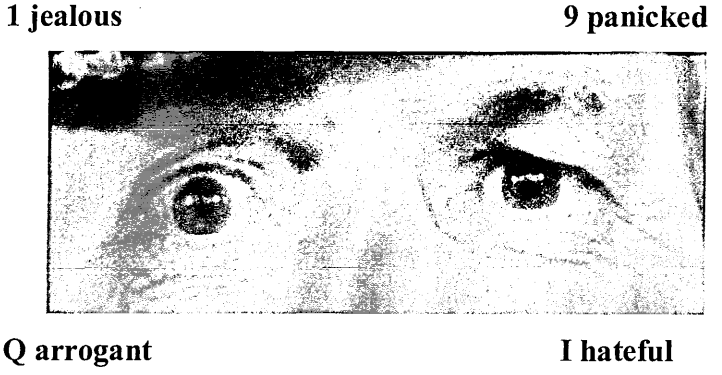
Multiplex and simplex parents completed three tasks; the Mind in Eyes task, the KDEF task and the EFT (see chapter three, sections 3.5.5-3.5.7). Parents also completed the Raven's Progressive Matrices and the Mill-Hill Vocabulary Scale (see chapter three, sections 3.5.9 and 3.5.10). Control participants also completed the KDEF and Mind in Eyes tasks online via the Cambridge University psychology website. Proband diagnoses were verified using the 3Di-Short and the ADOS-G (see chapter three, sections 3.5.11-3.5.12 and 3.6 for further details).

For the Mind in Eyes task, participants were shown 36 photographs of the eye region of people's faces on a computer screen and asked to choose the correct mental state word

[32] Numbers in superscript indicate total number of people within sample that failed to complete measure or to provide appropriate information in each sample. Only data that is analysed in section 5.5 is shown. KDEF: Karolinska Directed Emotional Faces; EFT: Embedded Figures Task.

from a choice of four that best describes what the person in the photograph is thinking or feeling (see Figure 5.1 for an example of stimuli used). Again, participants were given 20 seconds to answer each question and told to answer as accurately as possible. Participants were given a practice item first to ensure that they understood how to complete the measure. Results provided a total accuracy score (number of items chosen correctly) and a mean response time (for correct trials only).

Figure 5.1: *Example of Stimuli used in the Mind in Eyes task. Participants had to choose which mental state term best described what the person in the picture was thinking or feeling. The numbers and letters refer to the buttons that participants were instructed to press on the computer keyboard.*



The procedure for the KDEF task is described in chapter two (section 2.3.2). To reiterate, participants observed 140 photographs of faces on a computer screen expressing one of six basic emotions as well as a neutral expression. For each photograph, participants had to choose which word from a list of seven best described the expression in the picture. Participants were told they had 20 seconds for each photograph and so should answer as quickly and accurately as possible. Participants were given a practice item first to ensure

that they understood how to complete the measure. Results provided a mean response time across correct trials for each emotion category as well as the number of items chosen correctly for each emotion category.

Finally, the test administration procedure for the EFT followed that of Jolliffe and Baron-Cohen (1997). Participants were told they were going to be shown a series of complex and simple designs and the aim was to locate the simple designs within the complex ones (see Figure 5.2 for an example of stimuli used). Participants were shown 12 laminated cards in total, presented in a fixed order; on each card a complex design was displayed. For each item, participants were firstly given one of these cards for 15 seconds and asked to study it carefully. This card was then removed and they were given a second card for 10 seconds which displayed a simple design. Participants were again asked to study this design carefully. This card was then removed by the examiner and participants were told they would be shown the original complex design again and their job was to locate the simple design within the complex one. Once participants had found the simple design they were instructed to tell the examiner and trace around it with a wooden stylus. Participants were allowed to see the simple shape again for 10 seconds if requested to ensure that their performance on this measure was not confounded by working memory. Participants were given a practice item first to ensure that they understood how to complete the measure. A stopwatch was used to measure response time, which began as soon as the complex designs were reintroduced. Timing was stopped if participants wanted to see the simple shape again. Timing was continued if the participant had failed to trace around the simple shape correctly. The time was recorded if the participant

correctly traced around the simple shape. Participants had a limit of 180 seconds for each item and were told to answer as quickly as possible, but without making mistakes. If participants could not find the simple shape within 180 seconds then the item was marked as a failed response and a response time was not recorded. Therefore, in line with previous research using the EFT, two dependent variables were used for this measure; number of items correct (maximum = 12) and the mean response time for correct items only (in seconds). For extended details about the procedure, see Jolliffe and Baron-Cohen (1997).

Figure 5.2: *Example of Stimuli used in the EFT. Participants had to locate the simple design (a) embedded within the complex design (b).*

(a) **(b)**

5.4.3 Statistical Analyses

5.4.3.1 Dependent variables selected

Total accuracy score was selected as the dependent variable to analyse performance on the Mind in Eyes task. Most previous studies examining performance on this task have used accuracy scores as the dependent variable (e.g. Baron-Cohen and Hammer, 1997; Dorris et al., 2004). A response time measure was not used to assess performance on this task because, unlike the KDEF, the accuracy scores already show a normal distribution and are therefore sensitive to subtle differences in performance. Moreover, the time taken for participants to read and comprehend these words describing the mental state terms are likely to be strongly influenced by their verbal IQ. Whilst it is expected that accuracy scores will also be influenced by verbal IQ, it is predicted that the main factor of interest (group) will account for a significant proportion of the variance of accuracy scores independently of this confound, similar to what has been found previously (Dorris et al., 2004).

In contrast, two dependent variables were selected to analyse KDEF performance: accuracy scores and accuracy-adjusted response times for each emotion category (happy, sad, angry etc.). Accuracy-adjusted response times were calculated by dividing the mean response time for correct items by the fraction of items answered correctly for each emotion category. Using accuracy as a dependent variable is in line with previous research on facial emotion recognition (see chapter two), whilst accuracy-adjusted

response time was used as a potentially more sensitive and informative measure of facial emotion recognition performance than accuracy or response time alone (see chapter two for further details). By choosing to exmaine recognition performance for each separate emotion category, this analysis follows the 'discrete' rather than the 'dimensional' model of basic emotion recognition. The discrete model posits that basic emotions should be conceptualised as discrete categories rather than being united together by common underlying dimensions such as valence and arousal (see Hamann, 2012 for a review).

Finally, two dependent variables were selected to investigate performance on the EFT, in line with previous research (De Jonge et al., 2006; Happé et al., 2001); total accuracy (number of items correct) and response time per correct item. Previous studies have reported differences between individuals with ASC and controls or ASC parents and controls on at least one of these dependent variables (De Jonge et al., 2006; Happé et al., 2001; Ropar and Mitchell, 2001). Therefore, using both accuracy and response time enabled the results here to be directly compared with previous studies.

5.4.3.2 Outliers

On the Mind in Eyes task, all accuracy scores were located within 3 standard deviations from the mean. On the KDEF, one accuracy score and one mean accuracy-adjusted response time was located over 3 standard deviations from the overall mean. These data points were from the same participant; a father from a simplex family. Records from the testing day indicated that the father was extremely distracted during the test by his

daughter who disrupted the father's performance. It was therefore decided that this data was too unreliable to be included in this data set and so was excluded. All other data points were located within 3 standard deviations from the overall mean. Finally on the EFT, there were 4 accuracy scores located 3 standard deviations below the overall mean; however, these scores were not due to test administration problems or measurement errors and so these data points were kept in the data set. Finally, all mean response times on the EFT were located within 3 standard deviations of the mean.

5.4.3.3 Statistical Tests

Distributions of accuracy scores on the Mind in Eyes task did not significantly differ from a normal distribution with the exception of multiplex parents (Kolmogorov – Smirnov test: $D(62) = 0.13$, $p < 0.05$). Since distributions broadly conformed to a normal distribution, parametric tests were carried out on accuracy scores. Previous studies implicate/ emphasise the importance of controlling for verbal IQ on this test (Dorris et al., 2004; Peterson and Miller, 2012). Indeed, there was a significant positive correlation between verbal IQ and accuracy scores in our sample ($r = 0.30$, $p < 0.01$). Therefore, it was important to control for this variable in data analyses. As a result, multiplex parents were compared with simplex parents only, which allowed verbal IQ to be used as a covariate. The control group had not completed a test of verbal IQ so this variable could not be controlled for in this sample. By including verbal IQ as a covariate, one can determine the proportion of the variance in test scores that can be attributed to group and sex, independently of this variable. Performance on the Mind in Eyes task was analysed

by carrying out a 2-way ANCOVA on total accuracy score with group and sex as the between-subject factors and verbal IQ as the covariate.

The distribution of accuracy scores for each emotion category on the KDEF task displayed strong ceiling effects; there was extremely high negative skew on all emotion categories except facial expressions of fear. Distributions of accuracy scores therefore deviated significantly from a normal distribution. Transformations did not convert the data into normal distributions. Therefore, performance on the KDEF task was analysed by carrying out non-parametric Kruskal Wallis tests on accuracy scores for each facial emotion category (happy, sad, angry etc.) with group (multiplex parent, simplex parent and control) as the between-subject factor. Any significant differences were followed by three Mann Whitney tests (to compare all groups) with a Bonferroni correction for multiple comparisons. Sex differences in accuracy scores for each facial emotion category were also investigated by carrying out Mann Whitney tests with sex as the between-subject factor.

The distributions of accuracy-adjusted response times on the KDEF exhibited high positive skew and high kurtosis. Data was therefore logarithmically transformed to enable the use of parametric tests of statistical inference. There was a non-significant correlation between verbal/ non-verbal IQ and transformed accuracy-adjusted response time on the KDEF (both $p > 0.05$), so these measures were not used as covariates in data analyses. Therefore, one set of statistical analyses was carried out on this dependent variable, examining differences in performance between multiplex parents, simplex parents and

controls. KDEF task performance was investigated by carrying out a mixed ANOVA on transformed accuracy-adjusted response times with group and sex as the between-subject factors and emotion category (happy, sad, angry etc.) as the within-subjects factor. The mixed ANOVA could examine overall differences in performance on the KDEF test but could not assess possible differences on each individual emotion category. Therefore, the mixed ANOVA was followed up by a 2-way ANOVA on each emotion category with group and sex as the between-subject factors.

The distribution of accuracy scores on the EFT displayed strong ceiling effects (extremely high negative skew) and so significantly deviated from a normal distribution. Transformations did not substantially alter these distributions and so non-parametric tests only were carried out on this data. Performance was examined by carrying out a non-parametric Mann Whitney test on total accuracy scores with group (multiplex parent, simplex parent) and then sex as the between-subject factor. Distributions of response times were much closer to a normal distribution but some of these significantly deviated from normal. After data was logarithmically transformed, all distributions did not significantly deviate from a normal distribution except for simplex parents ($D(60) = 0.12$, $p < 0.05$). Non-verbal IQ correlated negatively with transformed response times on the EFT ($r = - 0.35$, $p < 0.001$), so this variable was used as a covariate in data analyses. A parametric 2-way ANCOVA was conducted on transformed response times, with group and sex as the between-subject factors and non-verbal IQ as the covariate.

5.5 Results

Table 5.2: *Descriptives for the Mind in Eyes task separated by group and sex.*

Group	Sex	Number of items correct (Max = 36)	
		Mean	SD
Multiplex Parent	Male (N= 30)	24.87	4.64
	Female (N = 32)	25.44	3.49
	Total (N = 62)	25.16	4.06
Simplex Parent	Male (N = 28)	26.11	4.18
	Female (N = 27)	27.74	4.41
	Total (N = 55)	26.91	4.33

Table 5.3: *Descriptives for the KDEF task separated by group and sex.*[33]

Group	Sex	Number of items correct per emotion category (Max = 20)			Mean ART (msecs) per correct item	
		Median	Mean	SD	Mean	SD
Multiplex Parent	Male (N = 28)	17.86	17.51	1.28	3115.9	675.4
	Female (N = 32)	17.43	17.33	1.08	3112.8	825.3
	Total (N = 60)	17.43	17.42	1.17	3119.6	752.8
Simplex Parent	Male (N = 28)	17.43	17.48	1.10	3168.6	762.6
	Female (N = 30)	18.21	18.06	0.94	2748.6	682.4
	Total (N = 58)	17.93	17.78	1.05	2951.4	746.4
Control	Male (N = 32)	17.57	17.36	1.24	3144.8	798.1
	Female (N = 32)	17.79	17.55	1.54	2896.9	709.7
	Total (N = 64)	17.64	17.45	1.39	3020.9	759.5

[33] KDEF: Karolinska Directed Emotional Faces; ART: Accuracy-adjusted response time; msecs: milliseconds.

Table 5.4: *Descriptives for the EFT separated by group and sex.*[34]

Group	Sex	Number of items correct (Max = 12)			Mean Response Time (secs) per correct item	
		Median	Mean	SD	Mean	SD
Multiplex parent	Male (N = 30)	12.0	11.17	1.05	24.02	14.57
	Female (N =32)	10.5	9.75	2.49	29.66	15.97
	Total (N = 62)	11.0	10.44	2.05	26.93	15.45
Simplex parent	Male (N = 30)	12.0	10.83	1.80	24.29	16.02
	Female (N = 30)	12.0	10.47	2.91	26.70	16.94
	Total (N = 60)	12.0	10.65	2.41	25.50	16.39

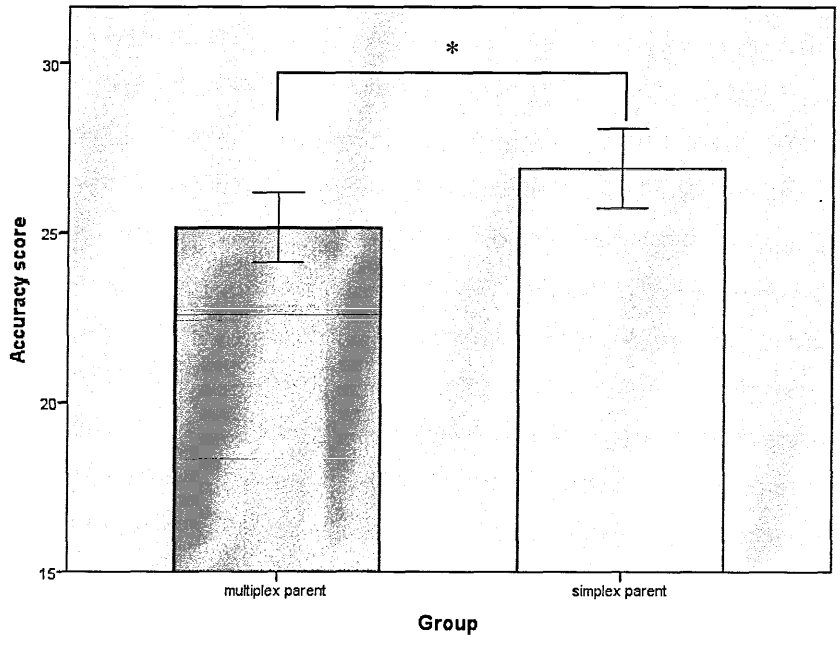
5.5.1 Complex emotion/ mental state recognition (Mind in Eyes)

Since Mind in Eyes test accuracy was found to be significantly associated with verbal IQ, performance on the Mind in Eyes task was only compared between simplex and multiplex parents, so that verbal IQ could be used as a covariate in the data analysis.

Results of a 2-way ANCOVA revealed a significant effect of the covariate on accuracy scores ($F(1,112) = 9.39$, $p < 0.01$, $r = 0.27$) and a significant main effect of group on accuracy ($F(1,112) = 4.24$, $p < 0.05$, $r = 0.17$), with multiplex parents scoring significantly lower than simplex parents (see Figure 5.3). The main effect of sex and the group × sex interaction were both non-significant ($p > 0.05$).

[34] Secs: seconds

Figure 5.3: *Main effect of group on accuracy (number of items correct) on the Mind in Eyes task.*[35]



5.5.2 Basic emotion recognition (KDEF)

5.5.2.1 Accuracy

Table 5.3 displays descriptives for the KDEF task, separated by group and sex. Accuracy scores for each emotion category were firstly analysed. Given previous findings of sex-specific expression of the BAP in first-degree relatives (Happé et al., 2001; Constantino et al., 2006), males and females were analysed separately. When males were analysed, the results of Kruskal Wallis tests indicated that the overall main effect of group was non-significant for all emotions (all $p > 0.05$). When females were analysed, the results of

[35] *$p < 0.01$. Error bars depict 95% confidence intervals.

Kruskal Wallis tests indicated that the overall main effect of group was significant for two emotions; happy ($H(2) = 7.41$, $p < 0.05$) and afraid ($H(2) = 9.47$, $p < 0.01$). These results were followed by three Mann Whitney tests each for happy and afraid expressions with a Bonferroni correction for multiple comparisons. These tests revealed that, contrary to predictions, control females were significantly less accurate at identifying happy facial expressions than multiplex mothers, which survived a correction for multiple comparisons ($U = 380.0$, $p < 0.01$). Contrast analysis also revealed that multiplex mothers were significantly less accurate than simplex mothers at identifying fear from facial expressions, which also survived a correction for multiple comparisons ($U = 261.5$, $p < 0.01$). No other significant differences were found.

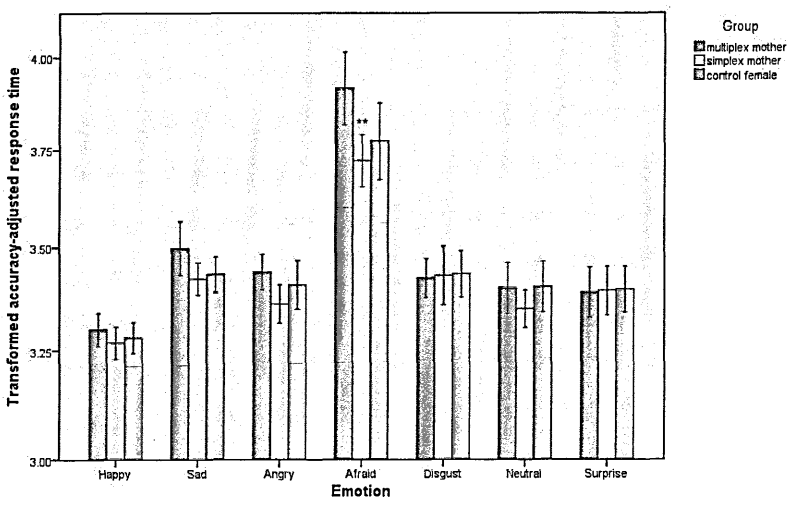
5.5.2.2 Accuracy-adjusted response time

Secondly, transformed accuracy-adjusted response times were analysed. Figure 5.4 displays a bar chart of the main effect of group on transformed accuracy-adjusted response times for each emotion category. The results of the mixed ANOVA revealed that the overall main effect of group was non-significant ($p > 0.05$), whilst the main effect of sex was significant ($F(1,176) = 5.36$, $p < 0.05$) with females outperforming males. The group × sex interaction was non-significant ($p > 0.05$). Results of within-subject effects also revealed a significant emotion × sex interaction ($F(3.65, 642.5) = 3.99$, $p < 0.01$) and a significant emotion × sex × group interaction ($F(7.30, 642.5) = 2.38$, $p < 0.05$). Results of contrasts between multiplex parents, simplex parents and controls were all non-significant ($p > 0.05$). Due to the significant emotion × sex × group interaction, this

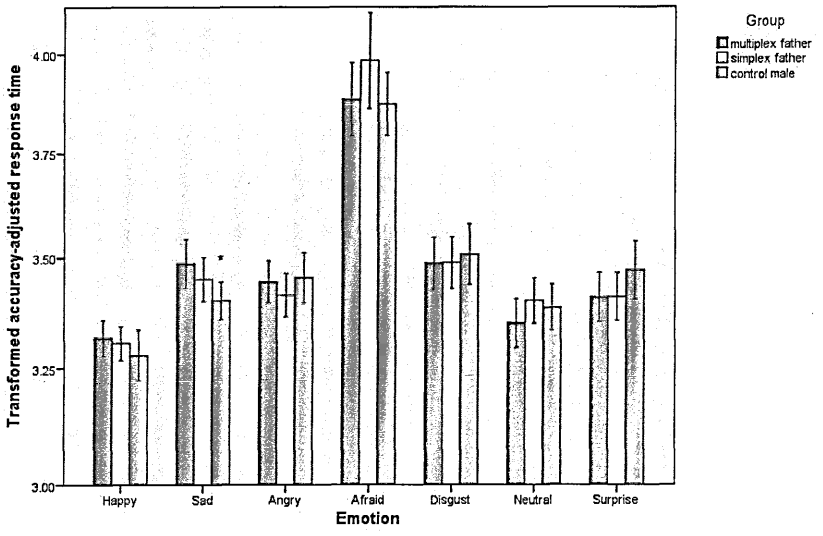
analysis was followed up with a series of sex-specific ANOVAs on each separate emotion category, with group as the between-subject factor. When only females were analysed, there was a significant main effect of group for just one emotion; afraid ($F(2, 91) = 4.96$, $p < 0.01$). Results of contrast analyses for this emotion indicated that the accuracy-adjusted response times of multiplex mothers were significantly higher than simplex mothers ($p < 0.01$, $r = 0.30$). This result remained significantly different after a Bonferroni correction for multiple comparisons. Results of contrast analyses also indicated significant differences between multiplex mothers and female controls for just one emotion; afraid ($p < 0.05$, $r = 0.23$). However, this result did not survive a correction for multiple comparisons. There were no significant differences between simplex mothers and female controls ($p > 0.05$). When only males were selected, there was a significant main effect of group for one emotion: sad expressions ($F(2, 85) = 3.12$, $p < 0.05$). Results of contrast analyses suggested that accuracy-adjusted response times were significantly higher in multiplex fathers compared to control males ($p < 0.05$, $r = 0.26$). This result survived a Bonferroni correction for multiple comparisons. No other significant differences were found between multiplex fathers, simplex fathers and male controls (see Figure 5.4). Therefore, the emotion × sex × group interaction seems to be partially driven by significantly worse performance recognising fear expressions in multiplex mothers (compared to simplex mothers), which is not found in multiplex fathers, and significantly worse performance recognising sad expressions in multiplex fathers (compared to male controls), which is not found in multiplex mothers (see Figure 5.4).

Figure 5.4: *Main effect of group on log-transformed accuracy adjusted response times for separate facial expressions of emotion on the KDEF: (a) females only, (b) males only.*[36]

(a)



(b)



[36] Significant differences between multiplex and simplex/ control groups denoted by the asterisks; *p < 0.05; **p < 0.01. Error bars depict 95% confidence intervals.

5.5.3. Attention to detail (EFT)

Table 5.4 displays descriptives for the EFT, including sample sizes, means and standard deviations. Firstly, non-parametric tests were carried out on accuracy scores. The results of a Mann Whitney test with group as the between-subject factor revealed a non-significant difference between multiplex and simplex autism parents ($p > 0.05$). Likewise, a Mann Whitney test with sex as the between-subject factor revealed a non-significant difference between ASC mothers and fathers ($p > 0.05$). Secondly, parametric tests were carried out on transformed response times. Results of a 2-way ANCOVA revealed non-significant main effects of group and sex (both $p > 0.05$) as well as a non-significant group × sex interaction ($p > 0.05$). The covariate, non-verbal IQ, had a significant influence on transformed response times ($F(1, 117) = 11.62$, $p < 0.01$, $r = 0.30$).

5.6 Discussion

This study is the first to examine differences in the cognitive profile of the BAP in multiplex versus simplex autism parents using tests of social and non-social cognition; on one of the former tests (the KDEF), multiplex and simplex parents were also compared to a control group. All groups were matched on age and education, whilst multiplex and simplex parents were also matched on verbal and non-verbal IQ. In particular instances, the study's predictions were supported but in the social domain only; firstly, multiplex mothers and fathers were significantly less accurate than simplex mothers and fathers at identifying complex mental states from the eye region of the face, after controlling for

verbal intelligence. Secondly, multiplex mothers, but not multiplex fathers, were significantly slower and less accurate than same-sex parents from simplex families at recognising facial expressions of fear, after controlling for a possible trade-off between accuracy and speed. Thirdly, multiplex fathers, but not multiplex mothers, were significantly slower than same-sex controls at recognising sadness from facial expressions, after controlling for a possible trade-off between accuracy and speed. In the non-social domain, the study's prediction was not supported; no significant group differences in performance were found for the EFT assessing attention to detail/ a local visual processing style. In all analyses there were no significant differences between simplex parents and controls. Therefore, these results suggest that the BAP may be expressed at a cognitive level in the relatives of probands from multiplex autism families for performance-based tasks in the social domain, including the recognition of complex emotions and mental states and possibly more basic negative valence emotions. The hypothesis of differential genetic mechanisms operating in multiplex and simplex autism (Constantino et al., 2010; Sebat et al., 2007) may apply with regards to the social cognitive phenotype of ASC/ the BAP. Each of the above findings from the study shall be discussed in further detail below.

The finding that multiplex parents have significantly poorer ToM ability, on average, than simplex parents adds to a small number of other studies that found significant differences in performance on the same ToM task (the Mind in Eyes) in the first-degree relatives of children with ASC versus controls (Baron-Cohen and Hammer, 1997; Dorris et al., 2004; Losh and Piven, 2007; Losh et al., 2009). Baron-Cohen and Hammer (1997),

Losh and Piven (2007) and Losh et al. (2009) reported this finding in parents, whilst Dorris et al. (2004) reported the finding in siblings. This study extends these results by suggesting that this performance indicator of milder ToM difficulties is mainly attributable to the first-degree relatives of probands from multiplex autism families. In addition to this result, the covariate, verbal IQ, had a significant effect on accuracy scores, which emphasises the importance of controlling for the influence that verbal intelligence has on the number of items participants answer successfully on this task. Previous studies matched groups on verbal intelligence (or a related measure), but not all studies used this variable as a covariate in data analyses (e.g. Baron-Cohen and Hammer, 1997; Gokcen et al., 2009). The study here suggests that being the first-degree relative (parent) of an autistic proband from a multiplex or simplex family has a significant effect on task accuracy after fully controlling for verbal intelligence.

The second and third findings suggest that multiplex mothers/ fathers perform significantly worse than same-sex parents from simplex families or same-sex controls at recognising specific, negative basic emotions from facial expressions. This is similar to a number of previous studies assessing basic emotion recognition in people with ASC and their first-degree relatives (Ashwin et al., 2006; Losh et al., 2009; Wallace et al., 2010). Wallace et al. (2010) reported that parents and adult siblings of people with ASC were significantly less accurate at identifying fear and disgust from facial expressions, whilst Losh et al. (2009) reported that parents displaying the 'social BAP' (aloof personality) were significantly less accurate at identifying fearful faces on the 'Morphed Faces' test, but only when this emotion was most faintly expressed. The latter result suggests that the

basic emotion recognition difficulties present amongst relatives of people with ASC are subtle and so tests need be designed with sufficient sensitivity to detect these differences (see chapter two). In the basic emotion recognition test reported here, sensitivity was increased by deriving an accuracy-adjusted response time, which is less vulnerable to displaying ceiling effects compared to other dependent variables such as accuracy scores. In contrast to the study reported in chapter two, the ASC parent group was here stratified into multiplex and simplex groups, which may have increased the test's power to detect subtle differences in first-degree relatives. When stratifying the ASC parent sample, results suggest that mothers from multiplex autism families are significantly slower and less accurate than mothers from simplex autism families at recognising facial expressions of fear. In contrast multiplex fathers, but not multiplex mothers, were significantly slower than same-sex controls at recognising a different basic negative emotion; sadness. Whilst previous studies have largely reported difficulties recognizing fear and disgust in first-degree relatives (Losh et al., 2009; Wallace et al., 2010), one study has also reported difficulties identifying sad facial expressions compared to controls (Palermo et al., 2006).

The results of task performance on the KDEF across the three groups indicate sex-specific effects recognising basic negative emotions in multiplex parents. A number of previous studies on the BAP have found evidence for sex-specific BAP characteristics, namely in male first-degree relatives (e.g. De la Marche et al., 2012; Happé et al., 2001; Virkud et al., 2009). Only one previous study has found a female-specific BAP; Groen et al. (2012) reported an atypical visual scanning pattern in mothers but not fathers of children with ASC. In the only previous study to examine performance on a test of social

cognition in multiplex versus simplex autism relatives, Bölte and Poustka (2003) reported superior performance in simplex autism siblings and parents on a test of basic facial emotion recognition. These differences between multiplex and simplex relatives were not sex-specific, although the researchers only used overall accuracy scores as the dependent variable and didn't explore group differences in recognising specific emotions. Nevertheless, similar results were found upon splitting the ASC relatives group into multiplex and simplex groups. More studies are needed exploring sex-specific effects on perceptual and social cognitive tasks in multiplex versus simplex autism relatives and controls. The significant sex-specific results here for recognition of facial expressions of fear and sadness perhaps suggests that the presentation of the cognitive BAP is slightly different in mothers and fathers of children with ASC.

A final unexpected finding on the KDEF task of basic emotion recognition was that control females were significantly less accurate at identifying happy expressions compared to multiplex mothers. This result was especially surprising because happy facial expressions are the easiest items to recognise, with the distribution of accuracy scores for happy expressions showing strong ceiling effects. The reasons behind this result are unclear; the mean accuracy-adjusted response time for happy items was slightly lower in control females compared to multiplex mothers, perhaps suggesting that controls tended to follow a different strategy that focused more on speed rather than accuracy. All control participants completed the KDEF test online without the presence of a test administrator, which may have lowered motivation and concentration in these participants, leading to this significant result. Whatever the reason, the finding suggests

that the results of comparisons between multiplex/ simplex parents and controls should be treated with caution. Further studies are needed using a control group that includes parents of typically developing children only, and who have completed the task in the same testing environment (offline, in the presence of a test administrator), and been recruited in a similar way to the multiplex and simplex parent groups (see chapter three).

In light of the above findings on tests of social cognition, it can be speculated whether these significant difference in performance involve differential functioning of brain regions recruited to complete these tasks in the parents of probands from multiplex autism families. Firstly, previous fMRI studies of the Mind in Eyes task have implicated high activity in a number of brain areas, including regions making up the 'social brain', such as the amygdala, medial prefrontal cortex, inferior frontal gyrus and superior temporal gyrus (Adams et al., 2009; Adolphs et al., 2002; Baron-Cohen et al., 1999; Moor et al., 2012). Poorer mean performance on this task in multiplex parents may thus indicate abnormal functioning/ integration of these areas, which are heavily involved in mental state reasoning. One can also speculate whether significant differences in performance recognising basic negative emotions between multiplex parents, simplex parents and controls reflect important neurofunctional differences. Whilst at least one study on basic emotion recognition in first-degree relatives examined overall performance only (Bölte and Poustka, 2003), the majority examined performance for separate emotions because there is evidence suggesting that the processing of different basic emotions involves separate neural substrates (Calder et al., 2001; Chakrabarti et al., 2006). Fear processing, including fear recognition from facial expressions, is the most

studied of all basic emotions and has implicated a number of brain regions including the anterior cingulate cortex, orbitofrontal cortex and the amygdala (Adolphs et al., 1999, 2005; Calder et al., 2001). It can therefore be speculated that the significant differences found between multiplex and simplex mothers may indicate functional differences in these areas subserving fear recognition. The significant performance differences between multiplex fathers and controls may also reflect important neurofunctional differences in a number of brain regions; the results of fMRI studies into the recognition of sadness from facial expressions are somewhat inconsistent, but have implicated ventromedial prefrontal areas, the subgenual cingulate cortex, hypothalamus and amygdala (Adolphs and Tranel, 2004; Chakrabarti and Baron-Cohen, 2006; Chakrabarti et al., 2006).

The final finding from this study was that there were no significant differences in the predicted direction between multiplex parents and simplex parents on the EFT. Thus, multiplex parents did not show evidence of a cognitive superiority in the visual attention domain. Other studies have reported superior performance on the EFT amongst ASC parents (especially fathers) compared to controls (Baron-Cohen and Hammer, 1997; Happé et al., 2001) but there have been no studies to date comparing multiplex and simplex relatives. These results do not support the hypothesis of differential genetic transmission in multiplex and simplex autism in this non-social perceptual domain. It also suggests that if there is a BAP for local processing style, it is not restricted to multiplex relatives; however, it should be noted that these groups could not be compared to controls for this task. The mean response times reported for ASC parents in this study are lower than those reported by De Jonge et al. (2006) who used the same response time measure

(mean response time for correct items only), and is similar to adults with ASC who have demonstrated superior performance on this task (25.9 seconds reported by De Jonge et al., 2006). The discrepancies between the response times reported here and by De Jonge et al. (2006) could be due to differences in test administration or measurement error. Previous studies also report accuracy scores for this measure; whilst accuracy is less vulnerable to measurement error, it may not be a sufficiently sensitive measure of test performance because of high ceiling effects. Therefore, there could be a number of confounding variables that are affecting scores on the EFT. White and Saldaña (2011) suggest a number of reasons for inconsistent results on this task, including using participants with different general ability, using different procedures to match groups and using different administration procedures and techniques. Despite these problems, the ostensibly fast response times and high accuracy scores displayed by both multiplex and simplex parents may represent superiorities in this domain by both sets of parents compared to a control group. However, it was not possible to examine whether this was the case because the control group used here had not completed the EFT.

Since the control group did not complete measures of general cognitive functioning it was not possible to match this group on IQ and control for verbal or non-verbal IQ during data analyses, so it is important to investigate cognitive differences between multiplex parents and controls further in new samples, using control participants who have completed a reliable measure of verbal and non-verbal IQ. Nevertheless, the study reported here provides new findings suggesting that a BAP at the cognitive level may be found in the first-degree relatives of multiplex autism families, but in the social domain

only, namely for emotion and complex mental state perception. These cognitive characteristics are associated with the reciprocal social interaction and communication impairments characterising clinical ASC. As such, these subtle difficulties may be possible cognitive endophenotypes for ASC (see chapter eight, section 8.5.1). However, it is important that future work establishes whether multiplex autism parents are significantly worse than appropriate controls on these social cognition tests after carefully matching both groups on IQ.

In this chapter and the last, the BAP has been examined from a behavioural and cognitive level, using self-report scales and performance-based tasks. In the next chapter, the parents of multiplex and simplex autism families are assessed for clinical and sub-clinical features associated with other psychiatric conditions in order to explore possible differences in psychiatric history amongst the relatives of multiplex and simplex probands.

Chapter Six

Exploring psychiatric history and parental psychopathology in Multiplex versus Simplex Autism Families.

6.1 Abstract

Previous studies indicate that a number of psychiatric problems aggregate in the genetic relatives of people diagnosed with ASC, including depression, anxiety, social phobia and Attention Deficit/ Hyperactivity Disorder (ADHD), which may represent an overlapping genetic liability with ASC. This study used a self-report questionnaire (the Adult-Self-Report Form; ASR) that quantitatively measures a wide range of psychiatric problems, to explore differences between parents from multiplex and simplex autism families. Parents also reported on their family's psychiatric history during a parental interview for ASC (the 3Di developmental dimensional and diagnostic interview). Results revealed significant aggregation of ADHD traits in the clinical range in multiplex parents compared to simplex parents ($p < 0.05$). Multiplex mothers, but not fathers, also reported significantly higher scores than simplex mothers on the somatic complaints scale of the ASR ($p < 0.05$). Compared to normative samples, both multiplex and simplex parents scored higher on most ASR scales; this was especially true for multiplex parents (notably mothers) for depressive problems, avoidant personality problems and Attention Deficit/ Hyperactivity (AD/H) problems. Finally, analysing reports of psychiatric problems in the family reported during the 3Di interview suggested that ADHD was more common in male siblings from multiplex families compared to male siblings from simplex families. Overall, results suggest high rates of a wide range of psychiatric problems in both simplex and multiplex autism families compared to a normative sample. Familial aggregation of ADHD traits in multiplex autism families compared to simplex families fits in with previous twin and family studies suggesting a genetic link between ADHD

and autism. A promising avenue for future research would be studies using ADHD families as a comparison group, to investigate cross-syndrome endophenotypes for ASC and ADHD.

6.2 Introduction

A number of research studies that have explored the BAP in the first-degree relatives of autistic probands have not only looked at milder characteristics of the autism phenotype, but also the full range of other conditions that are associated with a liability to ASC (e.g. Bolton et al., 1998; Piven and Palmer, 1999). These studies have consistently suggested that the relatives of individuals diagnosed with ASC are at an increased risk for other psychiatric problems. However, the specific psychiatric problems that aggregate in autism families have varied across studies, with mixed findings for most conditions. Strongest support has been found for depression (Bolton et al., 1998; Ingersoll et al., 2011; Micali et al., 2004; Piven and Palmer, 1999; Smalley et al. 1995), anxiety disorders (Piven et al., 1990, 1991; Piven and Palmer, 1999) and Obsessive Compulsive Disorder/ related traits (Wilcox et al., 2003; Hollander et al., 2003) (see chapter one, section 1.3.3 for a review). In all previous studies, the first-degree relatives of autistic probands were compared to the first-degree relatives of either typically developing children (Gold, 1993; Micali et al., 2004) or children with another disability (e.g. Down Syndrome; Bolton et al., 1998). Data collection procedures have varied, with some studies using semi-structured interviews about family psychiatric history (e.g. the Maudsley Version of the Schedule for Affective Disorders and Schizophrenia-Lifetime Version [SADS-L]; Piven and Palmer, 1999 and the FHI; Bolton et al. 1994, 1998) whilst others used self-report questionnaires that measured traits associated with other psychiatric conditions (e.g. Micali et al., 2004).

In this study, the parents of autistic probands from multiplex autism families are compared to those from simplex autism families using a quantitative self-report questionnaire measure of psychiatric problems. Multiplex parents are also compared to simplex parents on information they provided about family history of mental health problems in the 3Di developmental, dimensional and diagnostic interview (Skuse et al., 2004). For the first time, this study examines whether there is greater aggregation of psychiatric problems in the unaffected members of multiplex autism families compared to simplex autism families. Following the hypothesis of distinct genetic aetiology underlying autism in multiplex versus simplex families (as set out in chapter one, section 1.4.3), the unaffected members of multiplex autism families are thought to be more likely to carry a genetic liability for ASC. If there is greater aggregation of other psychiatric problems in multiplex families compared to simplex families, then this would suggest there may be overlap in the genetic aetiology between those problems and ASC/ the BAP. A small number of conditions have been suggested as potentially sharing overlapping genetic liability for ASC, including affective disorders (Bolton et al., 1998) and ADHD (Rommelse et al., 2011). By assessing whether there is aggregation of other psychiatric problems in multiplex autism families compared to simplex autism families, this study adds further evidence to support or refute the suggestion that other conditions may share common genetic aetiological factors with ASC.

6.3 Methods

6.3.1 Participants

The same sample of ASC parents used in chapters four and five were selected for this study: 60 simplex parents (30 mothers, 30 fathers) and 64 multiplex parents (32 mothers, 32 fathers). All parents completed the ASR offline, prior to the testing day, whilst parents provided information about psychiatric history in the 3Di interview during the testing day. Control data was not collected for the measures used in this study; however, mean scores and standard deviations on the ASR could be compared to normative samples in Appendix C of the Manual for the ASEBA Adult Forms and Profiles (Achenbach and Rescorla, 2003). ASC parents completed the Raven's Progressive Matrices (SPM+ version) and the Mill-Hill vocabulary Scale on the testing day. The simplex and multiplex parent groups did not significantly differ on non-verbal IQ (using the Raven's Progressive Matrices; $p = 0.74$), verbal IQ (using the Mill-Hill vocabulary scale; $p = 0.40$), age ($p = 0.74$) and education ($p = 0.38$). Proband diagnoses had been verified with the ADOS-G and 3Di-short (see chapter three). The majority of parents had completed the ASR and the 'family' section of the 3Di interview (see Table 6.1).

Table 6.1: *Descriptives for the ASR and 3Di interview-family section.*[37]

	Multiplex Parents	Simplex Parents
N	64	60
Mean Age (years) (SD)	44.5 (5.3) [1]	44.9 (7.3)
Non-verbal IQ (SD)	96.9 (11.4) [2]	97.6 (11.1)
Verbal IQ (SD)	98.3 (13.9) [2]	100.4 (13.4)
% with higher education qualification	52.5 [5]	60.7 [4]
% of sample completing ASR	98.4 [1]	96.7 [2]
% of sample completing 'family' section of the 3Di	96.8 [2]	100

6.3.2 Materials and Procedure

Parents completed the ASR (Achenbach and Rescorla, 2003). The ASR is a self-report questionnaire that measures people's perceptions of their own functioning. It can be split into 6 DSM-oriented scales where each scale includes items that have been rated by experienced psychologists and psychiatrists as consistent with a DSM diagnostic category. These include traits consistent with the following DSM categories: Depression, Anxiety disorders, Somatic disorders, Avoidant personality disorder, Attention Deficit/ Hyperactivity Disorder (ADHD) and Anti-social personality disorder. For further details about this measure see chapter three, section 3.5.8.

In addition to parents self-reporting behavioural problems using the ASR, parents were also asked about psychiatric problems in the family during the 3Di developmental, dimensional and diagnostic interview (section 3: 'the family'; Skuse et al. 2004; see

[37] Numbers in superscript indicate total amount of people within sample that failed to complete measure or to provide appropriate information in each sample.

chapter three, section 3.5.11). Parents were asked if they have had any significant problems with mental health and the same question was asked about the proband's siblings. This information is useful in ascertaining whether other psychiatric problems occur more frequently within multiplex or simplex autism families, and determines whether there are any problems detected in the ASR that are not reported as a mental health problem in the interview, or vice-versa.

6.3.3 Statistical Analyses

The distribution of DSM-oriented scores on the ASR displayed high positive skew; a high number of participants registered low scores on each scale. As a result, most distributions significantly deviated from a normal distribution. Furthermore, transformations did not substantially improve the normality of the data distributions. Therefore, non-parametric statistical tests were conducted on this data, which consisted of a series of Mann-Whitney U tests, with group (multiplex parent versus simplex parent) as the between-subject factor. Using the Mann-Whitney test protected against the potential biasing effects of outliers because, unlike parametric tests, it does not analyse significant differences in mean scores; rather, it examines group differences in how data values are ranked. The gender ratio was approximately equal in both groups (male to female ratio: 28:30 in simplex families; 31:32 in multiplex families), so mothers and fathers were first analysed together to maximise power. This was followed by a more focused analysis that considered ASC mothers and fathers separately.

In addition to analysing group differences in mean scores across all mothers and fathers from multiplex and simplex autism families, a second analysis was conducted examining differences in the proportion of parents scoring in the clinical range on each DSM-oriented subscale of the ASR. Scores that fall above the 97th percentile of the normative sample for each subscale are considered high enough to be of clinical concern (Achenbach and Rescorla, 2003). Associations between family status and the numbers of parents scoring in the clinical range on the ASR subscales were examined using the Fisher exact test (2-tailed). For these comparisons only parents aged between 36 and 59 years were assessed because the clinical ranges provided by Achenbach and Rescorla (2003) differ between participants aged 18-35 and participants aged 36-59; the majority of parents in the current sample who completed the ASR were aged between 36 and 59 years (109 out of 121 participants).

Finally, the proportion of reported psychiatric problems in multiplex and simplex autism families during the 3Di interview were compared; firstly in parents, and then in siblings of the proband. Associations between family status and the number of parents reporting psychiatric problems in the family (sibling/ parent) were examined using the Fisher exact test (2-tailed). Some psychiatric problems are more prevalent in males or females (e.g. ADHD in males; Cuffe et al., 2001) and so it was necessary to analyse male and female siblings separately to ensure that sex was not confounding the results.

6.4 Results

6.4.1 DSM-oriented scales of the ASR (raw scores)

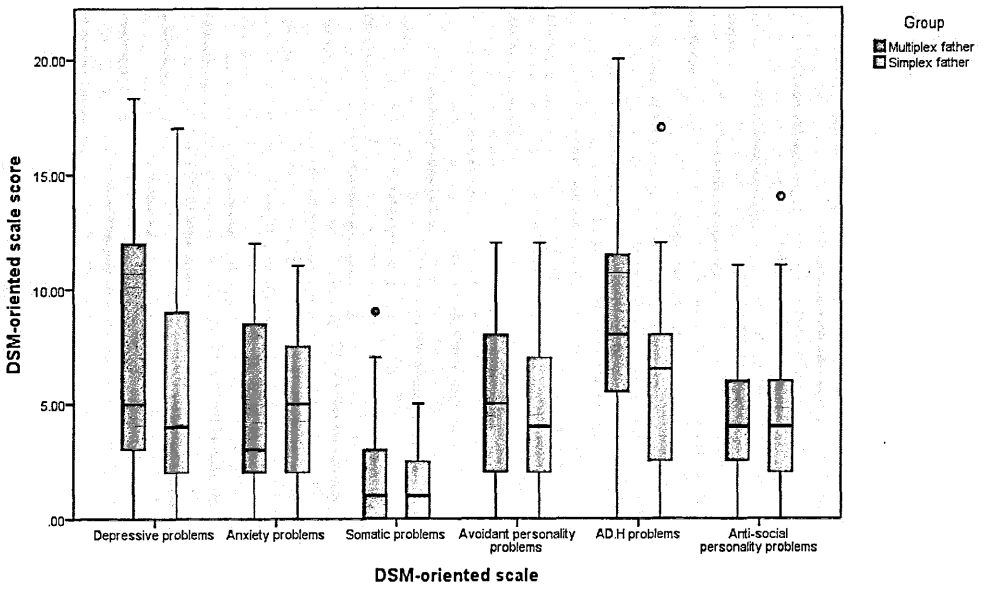
Descriptives for the DSM-oriented scales are provided in Table 6.2; these include means and standard deviations for an age-restricted sample (36-59 years of age), which enable comparisons to be made between these samples and normative samples provided by Achenbach and Rescorla (2003).

Mann Whitney tests were first carried out examining differences between multiplex parents (mothers and fathers) and simplex parents (mothers and fathers). These revealed a non-significant main effect of group on all DSM-oriented scales (all $p > 0.05$). AD/H problems approached significance ($p = 0.06$) where higher mean scores were reported by multiplex parents.

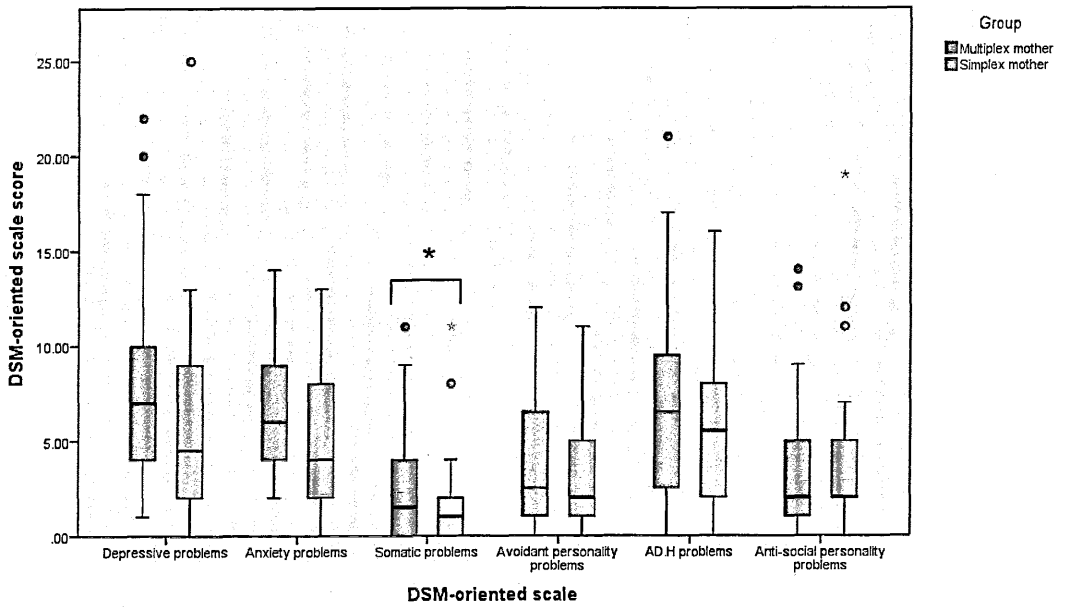
When ASC mothers and fathers were analysed separately, the results of Mann-Whitney U revealed that somatic complaints were significantly higher in multiplex mothers compared to simplex mothers ($U = 338.0$, $p < 0.05$). The AD/H problem scale approached significance in fathers ($p = 0.05$) and anxiety problems approached significance in mothers ($p = 0.05$), where higher mean scores were reported by multiplex parents. No other group effects were found for any of the other scales (all $p > 0.05$). The effect of group on DSM-oriented scores is displayed in Figure 6.1.

Figure 6.1: *The main effect of group on DSM-oriented scales of the ASR; (a) ASC Fathers and (b) ASC Mothers.*[38]

(a) ASC Fathers



(b) ASC Mothers



[38] Significant differences between multiplex and simplex parent groups denoted by the black asterisk: *p < 0.05. Outliers denoted by the coloured circles and asterisks.

Table 6.2: *Mean scores and standard deviations for ASR scales, including normative samples: (a) Males only and (b) Females only.*[39]

(a)

	Multiplex Father N= 31 *(N = 29)*		**Simplex Father N = 28** *(N = 25)*		**Male normative sample** *(N = 435)*	
	Mean	SD	Mean	SD	Mean	SD
DSM-Oriented Scale						
Depressive problems	6.8 *(7.1)*	5.6 *(5.6)*	5.9 *(6.1)*	5.1 *(5.3)*	*(3.2)*	*(3.0)*
Anxiety problems	4.9 *(5.0)*	3.8 *(3.9)*	5.1 *(5.2)*	3.1 *(3.3)*	*(3.7)*	*(2.5)*
Somatic problems	1.7 *(1.8)*	2.2 *(2.2)*	1.3 *(1.2)*	1.6 *(1.7)*	*(1.3)*	*(2.0)*
Avoidant personality problems	5.0 *(5.3)*	3.3 *(3.3)*	4.4 *(4.6)*	3.1 *(3.2)*	*(2.2)*	*(2.1)*
AD/H problems	8.3 *(8.5)*	4.8 *(4.7)*	5.9 *(6.1)*	4.1 *(4.2)*	*(4.5)*	*(3.6)*
Anti-social personality problems	4.3 *(4.4)*	2.7 *(2.8)*	4.4 *(4.4)*	3.2 *(3.4)*	*(3.0)*	*(3.0)*

(b)

	Multiplex Mother N = 32 *(N = 30)*		**Simplex Mother N = 30** *(N = 25)*		**Female Normative Sample** *(N = 621)*	
	Mean	SD	Mean	SD	Mean	SD
DSM-Oriented Scale						
Depressive problems	7.8 *(7.9)*	5.5 *(5.6)*	5.8 *(5.8)*	5.4 *(5.5)*	*(4.1)*	*(3.5)*
Anxiety problems	6.5 *(6.7)*	3.3 *(3.3)*	5.0 *(5.1)*	3.3 *(3.0)*	*(4.4)*	*(2.7)*
Somatic problems	2.4 *(2.2)*	2.7 *(2.5)*	1.8 *(1.7)*	2.5 *(2.3)*	*(1.7)*	*(2.3)*
Avoidant personality problems	4.1 *(4.3)*	3.7 *(3.7)*	3.3 *(3.4)*	3.1 *(3.1)*	*(2.5)*	*(2.1)*
AD/H problems	7.2 *(7.5)*	5.8 *(5.8)*	5.7 *(5.9)*	4.0 *(4.1)*	*(4.4)*	*(3.6)*
Anti-social personality problems	3.4 *(3.7)*	3.4 *(3.9)*	3.8 *(4.0)*	4.2 *(4.5)*	*(2.4)*	*(2.3)*

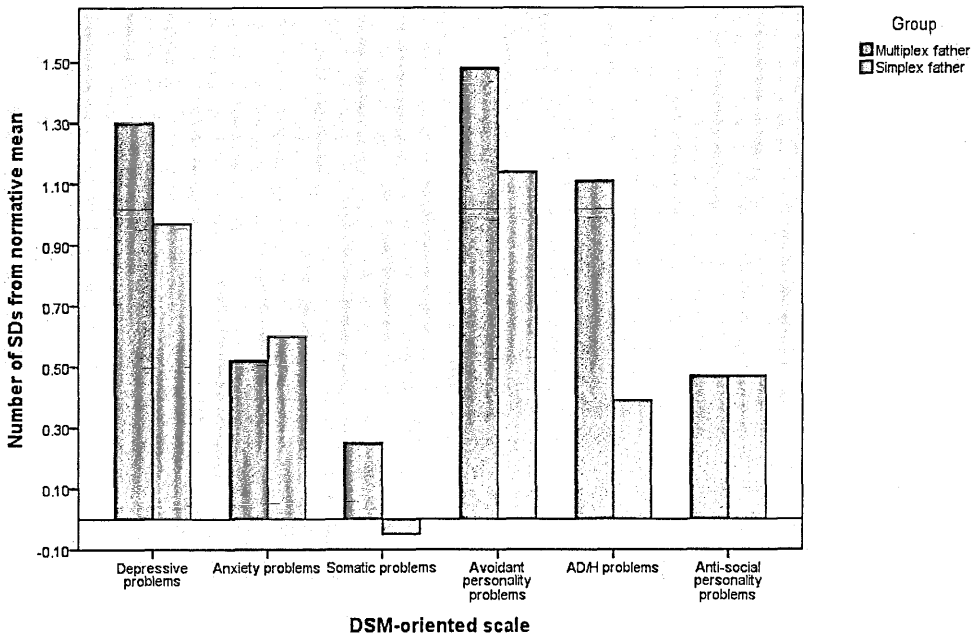
[39] mean and SDs for age category: 36-59 given in brackets.

6.4.2 Comparison of scores with normative data

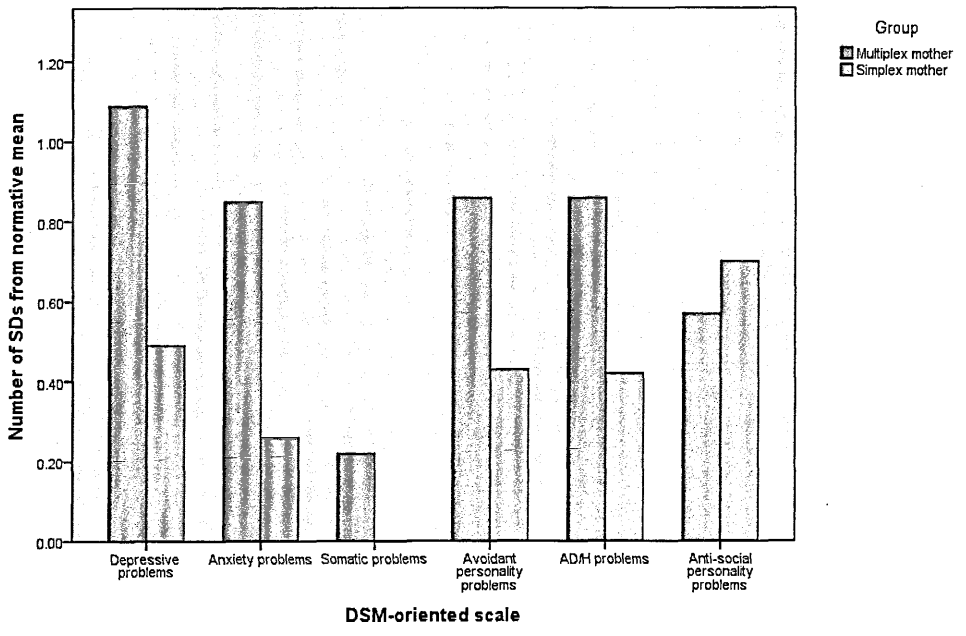
Scores on each ASR scale were compared to normative data to gain some insights into the extent to which scores from multiplex and simplex parents deviated from nonreferred normative samples (see Figure 6.2). In both multiplex and simplex fathers, mean scores on the DSM-oriented scales deviated above the male normative mean with the exception of somatic problems in simplex fathers. Scores were especially higher than the normative sample in multiplex fathers for the AD/H problem scale, and in both multiplex and simplex fathers on the scales: depressive problems and avoidant personality problems. Amongst multiplex and simplex mothers, scores on the DSM-oriented scales were notably higher than the normative sample in multiplex mothers, especially depressive problems, anxiety problems, avoidant personality problems and AD/H problems.

Figure 6.2: *Comparing multiplex and simplex parents to nonreferred normative samples on DSM-oriented scales of the ASR: (a) ASC fathers and (b) ASC mothers (age category: 36-59).*

(a) ASC fathers



(b) ASC mothers



6.4.3 Comparison of clinical scores on the DSM-oriented scales

The proportion of parents from multiplex and simplex families who scored in the gender-specific clinical range on each DSM-oriented scale of the ASR was compared using Fisher exact tests. These revealed a significant association between group and number of parents scoring in the clinical range on the DSM-oriented AD/H problem scale (p (two-tailed) < 0.05), with a significantly higher proportion of multiplex parents than simplex parents scoring in the clinical range on this scale (16.9% versus 4.0%). No other significant group differences were found (all p > 0.05). Table 6.3 displays the percentage of parents from each group that fell in the clinical range of scores on each DSM-oriented scale. Our sample sizes did not permit for analyses in males and females separately.

Table 6.3: *Percentage of multiplex and simplex parents scoring in the clinical range on the DSM-oriented scales of the ASR.*

DSM-oriented scale	**Multiplex Parents (N=59)**		**Simplex Parents (N=50)**		**Fisher exact P value (2 sided)**
	N	%	N	%	
Depressive problems	14	23.7	7	14.0	.23
Anxiety problems	9	15.3	4	8.0	.38
Somatic problems	3	5.1	1	2.0	.62
Avoidant personality problems	16	27.1	7	14.0	.11
AD/H problems	10	16.9	2	4.0	.04*
Anti-social personality problems	5	8.5	4	8.0	1.0

6.4.4 Family psychiatric history

Parents described psychiatric problems in the family during the 3Di parental interview ('Family' section; see methods); these included both clinically diagnosed problems and

possible problems that are of clinical concern. The problems reported were categorised and the proportion of parents from simplex and multiplex families reporting each problem were compared using Fisher exact tests (see Table 6.4). There were no significant associations between group and number of parents self-reporting a psychiatric problem (all $p > 0.05$). Both groups of parents self-reported high levels of depression (28.1% [multiplex] versus 26.7% [simplex]); a small number of parents also self-reported anxiety problems, whilst 6.3% of multiplex parents self-reported problems relating to dyslexia. Amongst siblings, there was a significant association between group and the proportion of parents reporting ADHD problems in male siblings (p (2-tailed) $< .05$), with parents reporting a significantly higher frequency of problems in the brothers of probands from multiplex families. No other significant associations were found (see Tables 6.5 and 6.6). It is noted that all ten male siblings from multiplex autism families with reported ADHD problems also had a co-morbid diagnosis of ASC. One limitation of this finding is that the data analysed included all available siblings per family in order to maximise power, but this meant that when multiple siblings per family were included, the data were not fully independent. Nevertheless, out of the 10 male siblings with reported ADHD, 9 came from separate families, so this finding cannot be attributed to the inclusion of many affected siblings from a single or very few families. Relative to multiplex siblings, the number of simplex brothers and sisters with a reported psychiatric problem was extremely low (see appendix 8 for the full tables of reported conditions). Greater than 5% of multiplex brothers were reported as displaying problems relating to dyspraxia, dyslexia and epilepsy. The highest frequencies of psychiatric problems

reported in multiplex sisters were depression and phobias, although sample sizes for these groups were modest.

Table 6.4: *Self-reported conditions in parents from multiplex and simplex autism families during the 3Di interview. Includes possible and definite disorders.*[40]

Reported condition	Multiplex Parents (N = 64)		Simplex Parents (N = 60)		Fisher exact P value
	N	%	N	%	
Depression	18	28.1	16	26.7	1.0
Anxiety	3	4.7	5	8.3	.48
Dyslexia	4	6.3	1	1.7	.37

Table 6.5: *Reported conditions in brothers of probands from multiplex and simplex autism families during the 3Di interview.*

Reported condition	Multiplex brothers (n = 33)		Simplex brothers (n = 17)		Fisher exact P-value
	N	%	N	%	
ADHD	10	30.3	0	0	0.01*
Dyspraxia	3	9.1	0	0	0.54
Dyslexia	3	9.1	0	0	0.54
Epilepsy	2	6.1	0	0	0.54

Table 6.6: *Reported conditions in sisters of probands from multiplex and simplex autism families during the 3Di interview.*

Reported condition	Multiplex sisters (n = 14)		Simplex sisters (n = 22)		Fisher exact P-value
	N	%	N	%	
Dyspraxia	1	7.1	0	0	0.39
Dyslexia	1	7.1	2	9.1	0.39
Depression	2	14.3	0	0	0.14
Pathological Demand Avoidance	1	7.1	0	0	0.39
Hypermobility	1	7.1	0	0	0.39
Phobias	2	14.3	0	0	0.14

[40] Only conditions with frequencies over 5% in at least one group is shown; for the full range of conditions reported in parents, male siblings and female siblings see appendix 8.

6.5 Discussion

The primary aim of this study was to examine differences between multiplex and simplex autism parents on a self-report measure of psychiatric problems and to compare these results to parental reports of psychiatric problems in the family during the 3Di developmental, dimensional and diagnostic interview. A significant association was found between multiplex/ simplex autism family status and the number of parents reporting AD/H problems in the clinical range; a significantly higher percentage of multiplex parents than simplex parents self-reported AD/H problems in the clinical range. When all raw scores on each ASR scale were examined, a significantly higher incidence of somatic complaints were reported by multiplex mothers compared to simplex mothers, although the overall number of multiplex parents reporting high scores on this scale was low (e.g. only three parents scored in the clinical range). There were no significant differences between simplex and multiplex parents on any other ASR scale, including problems associated with depression, anti-social personality disorder and anxiety, although AD/H approached significance in males and anxiety approached significance in females. Comparing the proportion of multiplex parents and simplex parents on scores in the clinical range, we found that a significantly higher number of multiplex parents (17%) than simplex parents (4%) scored in the clinical range of self-reported AD/H problems. Compared to normative samples, scores from both multiplex and simplex parents were qualitatively much higher, particularly depressive problems and avoidant personality problems in males and depression, anxiety and avoidant personality problems in multiplex mothers. Reporting of psychiatric problems in the family during the 3Di

interview did not reveal significant differences between multiplex and simplex families in the aggregation of psychiatric problems in the parents themselves, but there was a reported aggregation of ADHD in male siblings from multiplex families. However, it is acknowledged that the application of simplex/ multiplex criteria did not permit the inclusion of simplex families with 'unaffected' siblings above a clinical threshold on the Autism-Spectrum Quotient, which may have removed some provisional simplex families containing siblings with comorbid psychopathology, including traits consistent with ADHD. Only three simplex families were removed due to this criterion however (see chapter three; section 3.7) and so the exclusion of these families are unlikely to have significantly altered the results. Future studies examining psychiatric problems in ASC siblings would benefit from studies with larger sample sizes, applying techniques that correct for the problem of data non-independence in siblings using structural equation modelling. In summary, results provide some evidence for the aggregation of ADHD problems in two different first-degree relatives from multiplex families (siblings and parents) using two different measures (self-report questionnaire and parental interview).

The significantly higher incidence of ADHD problems in the parents and siblings of multiplex versus simplex autism families provides some support for the hypothesis that ASC and ADHD share overlapping genetic origins (Rommelse et al., 2011). The hypothesis that ASC and ADHD share partially overlapping underlying dimensions of liability has not been extensively tested, but some support is provided by Ronald et al. (2008) who found genetic correlations > 0.5 between autistic and ADHD traits in twins using a sample from the general population; this remained the case when only

participants reporting extreme scores on the behavioural measures were analysed. The findings reported here suggest it would be useful to compare ASC and ADHD groups in future studies using various behavioural, cognitive and neuroimaging measures to test for potential cross-syndrome endophenotypes. Rommelse et al. (2011) have suggested using these two clinical groups to test a number of endophenotypes, including language (pragmatics), executive function, face processing/ emotion recognition, arousal and reward in response to social stimuli, sustained attention and sensory functioning. Rommelse et al. (2011) argue that these domains hold the greatest potential for cross-syndrome cognitive endophenotypes because: (1) there is some evidence to suggest that impairments in these domains are related to both conditions, (2) they have distinct neural correlates and: (3) they have been shown to be heritable. In addition, another important criterion is that the suggested endophenotype should be found in the unaffected relatives of people with the clinical condition at a higher rate than the general population. With regards to ASC, the review of the BAP provided in chapter one indicates that pragmatics and face processing/ emotion recognition hold strongest support for meeting this criterion, with weaker support for executive function. No studies have examined social motivation, sustained attention and sensory functioning in ASC relatives. Therefore, it is clear that much more research is needed to gain further insights into whether these suggested domains hold promise for cross-syndrome endophenotypes (see chapter eight, section 8.5.1 for a further discussion of endophenotypes).

Results also support previous studies that report high rates of depression and anxiety in the parents of children with ASC (Bolton et al., 1998; Micali et al., 2004; Piven and

Palmer, 1999). Depression, anxiety and avoidant personality problems were all high in multiplex and simplex parents compared to normative samples. Although higher rates were especially seen in multiplex parents (particularly mothers), multiplex-simplex differences were not significant suggesting that these problems may not be strongly associated with the genetic liability to ASC. However, one possible reason for the lack of significant group differences on these scales is that it is not possible to perfectly assign simplex family status (see chapter three, section 3.7 for classification criteria); some families assigned simplex status may have been multiplex if more children had been born into the family and so some simplex families may still have some increased genetic risk that has not been detected based on this classification system (see also chapter eight; section 8.5.2). Another alternative explanation is that depression and anxiety problems may arise from the stress of caring for children with special needs. However, previous studies that have compared ASC parents with clinical control groups suggests that high rates of affective problems in ASC relatives such as depression and anxiety can not be fully attributed to parenting stress (e.g. Bolton et al., 1998). Further studies using clinical control groups are needed, preferably those that involve a disability without a genetic liability (e.g. Down Syndrome).

There are a number of limitations to acknowledge in this study. Firstly, due to time constraints it was not possible to assess other psychiatric problems in the family in greater detail. Parents were only asked if they had been affected by a mental health problem and likewise for their children. Further details about the mental health problem would have been extremely informative, such as the duration of the mental health

problem, the severity of the mental health problem, the timing of the onset of the mental health problem and asking the parent if something had triggered the mental health problem (e.g. death of a family or friend, divorce/ marital separation, diagnosis of the child). It would have been particularly useful to know whether the mental health problem preceded the birth/ diagnosis of the proband to ascertain whether the mental health problem may be associated with the stress of rearing a child with a disability. There are a number of interviews that could be used that examine psychiatric problems in the family more extensively, including the FHI (Bolton et al., 1994) and the SADS-L (Bolton et al., 1998; Piven and Palmer, 1999). However, as a pointer towards future investigations, the results from the ASR and the 3Di interview reported here do not suggest significantly greater aggregation of affective disorders/ problems in multiplex autism families compared to simplex autism families. Results instead provide stronger support for the aggregation of ADHD traits in multiplex autism families.

Despite its limitations, this study has the virtue of being the first to explore differences in the aggregation of other psychiatric problems in the first-degree relatives of autistic probands from multiplex and simplex autism families. To summarise: results suggest significant aggregation of ADHD traits in the clinical range in multiplex parents and a significantly higher number of multiplex brothers were reported by their parents as having clinical diagnoses of ADHD or ADHD problems that were of clinical concern. Thus, two separate lines of evidence point towards the aggregation of ADHD problems in multiplex autism families, compared to simplex autism families. These results implicate that ADHD and ASC may have overlapping aetiology in multiplex families especially;

the genetic vulnerability resulting in an increased risk for ASC in multiplex autism families may also predispose family members to an increased risk for ADHD. Future studies could examine what specific features may be shared between the two conditions by exploring cross-syndrome endophenotypes for ASC and ADHD (Rommelse et al., 2011). Furthermore, these results have potential clinical implications, suggesting that ADHD-related problems should be more closely assessed by clinicians in autism families, especially in multiplex families, including both probands and closely related relatives. The results of this study also suggest that the rates of other psychiatric problems are high in both multiplex and simplex autism families compared to a normative sample, including depression, anxiety and avoidant personality. The rates of psychiatric problems aggregating in ASC parents are therefore quite wide-ranging and are not restricted to AD/H problems.

The study reported in this chapter is the last to explore differences in the expression of autistic traits and related phenotypes between ASC relatives stratified according to their affiliation to multiplex or simplex autism families. To recap: in chapter four no significant differences were found between multiplex parents, simplex parents and controls using self-report scales of autistic traits and two related psychological constructs: empathy and systemising (Baron-Cohen et al., 2003; Baron-Cohen and Wheelwright, 2004). In chapter five, multiplex parents were significantly less accurate than simplex parents at attributing mental states from the eye region of the face and were significantly slower and, in multiplex mothers only, less accurate at identifying specific negative basic emotions from facial expressions compared to simplex parents / controls. No significant

group differences were found on a performance-based task assessing perceptual attention to detail. Finally, the study described in this chapter reported significant aggregation of ADHD behaviours in the parents and siblings of autistic probands from multiplex autism families compared to simplex autism families. One topic that has so far been left unexplored is the associations between different levels of analysis. In the next chapter, the final empirical chapter of the thesis, the multiplex and simplex parent samples are collapsed, and the relationships among autistic characteristics at a 'behavioural' and 'cognitive' level are explored. The chapter aims to discern whether, amongst parents of autistic probands, social and non-social autistic characteristics tend to aggregate together or appear in isolation.

Chapter Seven

Exploring the 'fractionable autism dyad': do social and non-social autistic traits and related cognitive phenotypes segregate or aggregate in the unaffected first-degree relatives of people with ASC?

7.1 Abstract

In this final empirical chapter of the thesis, a modification of Happé and Ronald's 'fractionable autism triad' hypothesis (2008) was investigated in the first-degree relatives (parents) of autistic probands by examining relationships between self-report and performance-based measures of autistic traits and related phenotypes associated with either the social or non-social domains of clinical ASC. Results provided partial support for separate aggregation of social and non-social autistic traits and related cognitive phenotypes in the mothers and fathers of children with ASC. Across all ASC mothers and fathers, social and non-social characteristics tended to aggregate both across and within domains, when measured using self-report scales, whilst aggregation across or within these two domains occurred less frequently when social and non-social characteristics were measured using performance-based tasks. Selected non-social characteristics aggregated across measurement type (self-report and performance-based task) in ASC fathers and mothers. In ASC fathers, characteristics aggregated within the social domain, including self-rated empathy scores and accuracy scores on tests of basic facial emotion recognition and complex mental state recognition. The aggregation or segregation of social and non-social characteristics of ASC was further explored in the mothers and fathers who self-reported the highest levels of autistic traits in either the social domain or the non-social domain. Social and non-social characteristics aggregated together in both of these groups when the measurement type was self-report, but aggregated less frequently when the measurement type was performance-based task. Strongest support for fractionation of autistic characteristics was found in ASC fathers with high autistic

traits in the non-social domain, where there was aggregation of high self-rated systemising and a strong detail-focused cognitive style, and a lack of an association with low empathising/ attenuated performance on social cognition tasks. These results imply that the fractionation of social and non-social characteristics of ASC may depend on the individual's sex and may be restricted to cases where the core autistic symptoms are associated with high systemising and a strong detail-focused cognitive style. Recommendations for future research include carrying out factor analytic studies of social and non-social measures of autistic traits in the parents of autistic probands at both a behavioural and cognitive level using larger sample sizes than achieved here, with an emphasis on investigating the separate aggregation of non-social and social characteristics in ASC fathers.

7.2 Introduction

In the previous four chapters, differences in the expression of the BAP were explored in multiplex versus simplex autism parents using a battery of self-report scales and performance-based tasks that measured autistic traits and related cognitive phenotypes. In this final empirical chapter of the thesis, we turn our attention to the relationships between these different measures *within* ASC parents, with a focus on exploring the associations between some of the social and non-social measures that are used to characterise both the clinical ASC phenotype and the BAP.

ASC is currently diagnosed on the basis of a 'triad of impairments' in social interaction, communication and restricted, repetitive behaviours and interests (APA, 2000; WHO, 1993). But how tightly bound are these three behavioural domains that describe the clinical presentation of ASC? In a paper by Happé and Ronald (2008), the authors addressed this outstanding question in autism research by persuasively arguing that the different domains of the ASC syndrome are 'fractionable' and have distinct causes at a genetic, cognitive and neural level. In support of this hypothesis, Happé and Ronald drew, among other evidence, on the finding that the different symptom domains associated with ASC can be observed/ measured in isolation amongst members of the general population and in the genetic relatives of people with ASC, where fragmented features of the clinical phenotype make up the BAP.

A number of family studies of ASC provide support for this hypothesis by reporting evidence of impairments in one area of functioning but not in another amongst the first/second-degree relatives of autistic probands. These studies have examined group differences in scores between ASC relative and control groups, either at a 'behavioural' level using self-report scales/ interviews or at a 'cognitive' level using performance-based tasks. For example, studies using self-report scales or interviews have reported evidence for social impairments in ASC relatives in the absence of non-social characteristics associated with restricted repetitive behaviours and interests (e.g. Bishop et al., 2004; Ruta et al., 2011; Wolff et al., 1988). Other studies using the FHI have reported communication impairment and/or social impairment and/or rigid repetitive behaviour in ASC relatives (Bolton et al., 1994; Piven et al., 1997a; Szatmari et al., 2000). Bolton et al. (1994) and Szatmari et al. (2000) found evidence of impairments both across domains and in a single domain only (defined as the 'narrow' and 'broad' lesser variant phenotype respectively). In contrast, Piven et al. (1997a) did not indicate the extent to which these different types of behavioural deficits appeared together or in isolation.

A number of studies have also investigated impairments and superiorities in multiple *cognitive* domains amongst the relatives of autistic probands. Some of these have reported ToM or facial emotion recognition impairments in the absence of a detail-focused cognitive style (also referred to as 'weak central coherence': Frith, 1989; Losh et al., 2009; Scheeren and Stauder, 2008). Baron-Cohen and Hammer (1997) reported ToM impairment and a detail-focused cognitive style in ASC parents, although the authors did

not state whether the same parents displayed strengths and weakness in these two cognitive domains or whether they largely appeared independently amongst different parents. A larger number of studies have compared either ToM or a detail-focused cognitive style to another cognitive domain: executive function, with some studies showing ToM impairments and executive dysfunction (Gokcen et al., 2009; Mosconi et al., 2010), whilst others have reported a detail-focused cognitive style in the absence of executive dysfunction (Bölte and Poustka, 2006). Lastly, executive dysfunction has been reported in ASC relatives in the absence of ToM impairment or a detail-focused cognitive style (Hughes et al., 1997; Piven and Palmer, 1997). Overall, these results indicate strong heterogeneity at a cognitive level, with cognitive impairments/ superiorities in different domains appearing either in isolation or in combination.

Whilst these studies have offered important insights, few have extensively examined the relationships between various behavioural (self-report/interview) and cognitive (performance-based task) measures of autistic traits and related cognitive phenotypes in ASC relatives, and few have examined whether these different aspects of the BAP are located in the same participants. One exception is Losh and Piven (2007), who reported a relationship between social behavioural characteristics (aloof personality, lower quality social relationships and impaired pragmatic language use), measured using three semi-structured interviews, and attenuated performance on a measure of social cognitive ability (the Mind in Eyes task). Thus, multiple characteristics associated with the social domain of impairment of ASC appeared to aggregate in ASC relatives across different measurement types (interviews and performance-based task). In contrast, ASC relatives

characterised as 'rigid' using the same interviews were not significantly less accurate on the social cognition task compared to controls and parents without this personality trait, suggesting that these social and non-social characteristics segregate. Together these results suggest that the social characteristics of ASC aggregate separately from the non-social characteristics in ASC parents, thus supporting the idea that these two domains fractionate.

In the revised edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), to be published in May 2013, ASC (referred to as 'Autism Spectrum Disorders' in the DSM-5) will be diagnosed on the basis of a 'dyad' rather than a 'triad' of impairments that coalesces social interaction and communication problems into one domain (the social symptoms) and keeps restricted repetitive behaviours and interests as the second domain (the non-social symptoms) (APA, 2012). In keeping with this revision, and earlier empirical work in this thesis that treats social interaction and communication as one domain, rather than two distinct ones, this study aimed to examine dyadic relationships between the social and non-social domains of ASC in the first-degree relatives of autistic probands at both a cognitive and behavioural level. Consistent with Happé and Ronald's fractionable autism triad hypothesis, it is expected that the social and non-social aspects of ASC will aggregate separately in ASC relatives. This profile would mirror what has been reported in the general population. For example, Hoekstra et al. (2008) examined the factor structure of the AQ in a large general population and student sample, identifying a two factor model split into a higher order 'social interaction' factor and a non-social 'attention to detail' factor that only correlated

with each other modestly. Likewise, Ronald et al. (2005, 2006a) examined autistic-like behaviour in 3000 twins in the general population, finding modest-low correlations between the social and non-social domains of impairment (social impairment/ communicative difficulties and restricted repetitive behaviour). Studying fractionation of autistic traits and related phenotypes in the first-degree relatives of autistic probands holds an advantage over general population samples because there is stronger reason to believe that the traits/ characteristics being measured are directly relevant to the clinical manifestations of ASC, given the individuals' genetic relationship to the autistic proband and the vast literature that exists on the BAP in ASC relatives (see Sucksmith et al., 2011 for a review). Studying the fractionation of autistic traits and related phenotypes in first-degree ASC relatives rather than clinical ASC samples is also advantageous because it avoids the circularity that is involved in studying whether symptom domains fractionate in a group that are diagnosed on the basis that difficulties in these domains co-occur.

The study here examines the hypothesis that the DSM-5 dyad of symptoms characterising clinical ASC fractionate in the first-degree relatives of autistic probands by discerning whether the social and non-social symptoms appear in isolation among the unaffected parents of autistic probands. This is achieved by exploring relationships between various measures completed by ASC parents that tap into the social and non-social facets of ASC (see section 7.3.2). The fractionable autism dyad hypothesis would predict that social and non-social characteristics aggregate within but not across domains in ASC parents. If the social and non-social characteristics of ASC aggregated across domains then these results would not be consistent with the hypothesis that the dyad of impairments is fractionable.

Aggregation of both social and non-social characteristics would more strongly resemble the ASC profile, where commonly these different aspects co-occur, as would be expected on the basis of their diagnosis, which is defined by both social and non-social behavioural characteristics.

7.3 Methods

7.3.1 Participants

After applying proband exclusion criteria, there were 132 parents (66 mothers, 66 fathers) available for data analysis. This sample was slightly larger than those used in chapters four to six because parents were not excluded for failing to meet simplex/ multiplex classification criteria. In this study no distinction was made between simplex and multiplex autism parents; all parents were analysed together. This is because the study was examining within-person correlations rather than group differences, and analysing all parents together increased the sample size, which increases statistical power.

7.3.2 Materials used and their categorisation into the social and non-social domains of ASC.

Parents completed the same measures as described in chapters four and five; these included three self-report questionnaires (the AQ, EQ and SQ-R) and three performance-

based tasks (the KDEF, Mind in Eyes and EFT). For further details about these measures see chapter three, sections 3.5.2-3.5.7.

The same dependent variables were used as in chapters two to five. The AQ social interaction factor subscale scores, summed EQ scores, KDEF (accuracy and ART scores) and Mind in Eyes accuracy scores fell within the social domain of ASC whilst the AQ attention to detail factor subscale scores, summed SQ-R scores and EFT (accuracy and RT scores) fell within the non-social domains of ASC (see Table 7.1).

Table 7.1: *Classification of measures into the social and non-social domains of ASC*[41].

SOCIAL DOMAIN		NON-SOCIAL DOMAIN	
Measure (variable)	**Description**	**Measure** (variable)	**Description**
1. Self-report		**1. Self-report**	
AQ (social interaction factor subscale score)	Self-rated social autistic traits	AQ (attention to detail factor subscale score)	Self-rated non-social autistic traits
EQ (sum score)	Self-rated empathy	SQ-R (sum score)	Self-rated systemising
2. Performance-based task		**2. Performance-based task**	
Mind in Eyes (accuracy)	Recognition of complex mental states	EFT (accuracy/ RT)	Perceptual attention to detail
KDEF(accuracy/ ART)	Recognition of basic facial expressions of emotion		

7.3.3 Comparing parents with and without high scores on the AQ.

The ten highest scoring mothers and fathers on the AQ factor subscales were separated from the rest of the sample; those parents scoring highest on the AQ social interaction

[41] RT: Response Time; ART: Accuracy-adjusted response time (transformed)

factor subscale were referred to as the 'high autistic traits (social)' group and those scoring highest on the AQ attention to detail factor subscale were referred to as the 'high autistic traits (non-social)' group. These groups corresponded to the top 16-17% of mothers/fathers on each AQ subscale; this percentage was close to the proportion of parents designated the BAP by Bolton et al. (1994) using a dichotomous measure (11%), and was lower than the percentage used by Bishop et al. (2004) to separate participants into those with and without the broader phenotype using the AQ (24%). All other parents were assigned 'low-medium autistic traits' group status. By taking the 10 highest scoring mothers and fathers on each subscale, high scores on the AQ could be sampled whilst at the same time allowing meaningful comparisons to be made with the 'low-medium autistic traits' groups. The AQ was used to assign group status because it is a well validated measure of autistic traits that can be split into a social and non-social factor (Hoekstra et al., 2008), thus enabling the possibility of assessing whether outcome measures associated with both the social and non-social domains of ASC aggregate separately in ASC mothers and fathers. The high autistic trait groups were not referred to as 'BAP+' in this study because in chapter four no evidence was found for significant differences between ASC parents and controls on the AQ. This finding contrasts with previous studies that have consistently reported that the AQ can detect the BAP in parents of autistic probands (Bishop et al., 2004; Ruta et al., 2011; Wheelwright et al., 2010).

7.3.4 Statistical Analyses

Mothers and fathers were analysed separately because there were a number of significant within-family spousal correlations in the datset (e.g. SQ-R (mother) vs. AQatt.det. (father); Spearman's $\rho = 0.3$, $p < 0.05$). These spousal correlations prevent tha data from being fully independent during correlation analysis if mothers and fathers are analysed together. Furthermore, it was also important to analyse mothers and fathers separately because a number of previous studies suggest that some BAP characteristics may be sex-specific (e.g. Happé et al., 2001; De la Marche et al., 2012; Scheeren and Stauder, 2008) and so male and female relatives of autistic probands may show different behavioural and cognitive profiles. Aggregation of autistic traits and related phenotypes in ASC parents were firstly analysed by running multiple correlations between the output measures from three self-report scales (AQ, EQ, SQ-R) and the sum scores on three performance-based tasks (Mind in Eyes, KDEF and EFT). In this analysis, measures were described as aggregating if they were found to significantly correlate with each other.

Full-scale correlations were followed by a comparison of scores between parents with a high number of autistic traits in the social/ non-social domains and parents with a low-medium number of autistic traits in the social/ non-social domains. The ten highest scoring fathers and mothers on the AQ social interaction factor subscale formed the high autistic traits (social) groups, with scores ranging from 114 to 125 in fathers (1.3-1.9 standard deviations above the mean), and 105 to 131 in mothers (0.9-2.1 standard deviations above the mean). The ten highest scoring fathers and mothers on the AQ

attention to detail factor subscale formed the high autistic traits (non-social) groups, with scores ranging from 31 to 37 in fathers (1.3-2.4 standard deviations above the mean), and 29 to 39 in mothers (1.0-2.7 standard deviations above the mean). Measures compared included three self-report scales (AQ, EQ and SQ-R) and three performance-based tasks (Mind in Eyes, KDEF and EFT). High versus medium-low autistic trait group comparisons consisted of multivariate analysis of co-variance tests (MANCOVAs) for data displaying normal distributions, with non-verbal and verbal IQ used as the covariates, whilst Mann-Whitney tests were run on one variable that displayed high ceiling effects and thus strongly deviated from a normal distribution (KDEF accuracy scores). If there was a significant difference between groups on a measure then the trait/ characteristic in question was considered to aggregate with high scores on a self-rated measure of autistic traits in either the social or non-social domain.

7.4 Results

7.4.1 Full-scale correlations between measures of autistic traits and related cognitive phenotypes.

Table 7.2 shows the correlation matrix between output measures from the three self-report scales and three performance-based tasks, separated by sex. There were a large number of significant correlations between self-report scales; in ASC mothers, all self-report scales/ subscales correlated with each other, including measures of social and non-social characteristics of ASC. In ASC fathers, 3 out of 6 correlations were significant including those between the EQ and the AQ social interaction factor subscale and

between total SQ-R score and the AQ attention to detail subscale. Thus, when the measurement type was self-report scale there was some evidence suggesting that non-social and social characteristics aggregated separately in ASC fathers, but there was no evidence that social or non-social characteristics aggregated separately in ASC mothers, rather, all self-report scales correlated with each other suggesting that these social and non-social characteristics aggregated within and across domains. In contrast, there were a smaller number of significant correlations between performance-based measures; in ASC fathers, 5 out of 10 correlations between performance-based measures were significant; these included two between social and non-social performance-based measures (Mind in Eyes accuracy versus EFT accuracy, and KDEF ART versus EFT accuracy). In ASC mothers, 4 out of 10 correlations were significant; one of these was between social and non-social performance-based measures (Mind in Eyes accuracy versus EFT accuracy). In both ASC fathers and mothers there were significant correlations among social performance-based measures (Mind in Eyes accuracy, KDEF accuracy and KDEF ART) but not among non-social performance-based measures (EFT accuracy and RT). Thus, the results of performance-based measure correlations suggest aggregation of social cognitive measures that occurs somewhat separately from non-social performance-based measures assessing attention to detail, especially in ASC mothers.

Lastly, self-report scales were correlated with performance-based measures, to examine whether social and non-social characteristics aggregate separately across measurement types. In ASC fathers, 3 out of 20 correlations were significant; two of these were between a social cognitive measure and the EQ, whilst one was between a non-social

cognitive measure and a self-report scale measuring non-social characteristics. Thus, there was some evidence of aggregation of selected social and non-social characteristics across measurement types in ASC fathers. In contrast, in ASC mothers, 1 out of 20 correlations between performance-based measures and self-report scales was significant; the significant correlation was between a non-social cognitive measure and a self-report scale of non-social characteristics (SQ-R versus EFT RT).

Table 7.2: *Correlations between output measures from three self-report scales and three performance-based tasks in parents of children with ASC; (a) fathers only and (b) mothers only.*[42]

(a)

		1	2	3	4	5	6	7	8	9
Self-report scale	1. AQ soc.									
	2. AQ att.det	**.359**								
	3. EQ	**-.795**	-.205							
	4. SQ-R	.208	**.523**	-.041						
Performance task	5. Eyes accuracy	-.162	.033	**.329**	.192					
	6. KDEF accuracy	-.215	.062	**.325**	.113	**.530**				
	7. KDEF ART	-.011	-.097	-.090	-.145	**-.408**	**-.516**			
	8. EFT accuracy	-.064	.182	.109	-.067	**.311**	**.252**	-.090		
	9. EFT RT	-.081	-.160	.027	**-.390**	-.096	-.179	.245	-.223	

(b)

		1	2	3	4	5	6	7	8	9
Self-report scale	1. AQ soc.									
	2. AQ att.det	**.514**								
	3. EQ	**-.814**	**-.331**							
	4. SQ-R	**.422**	**.512**	**-.309**						
Performance task	5. Eyes accuracy	-.074	-.009	.202	.038					
	6. KDEF accuracy	-.059	-.099	.097	-.102	**.494**				
	7. KDEF ART	-.106	-.114	.064	.043	**-.304**	**-.413**			
	8. EFT accuracy	-.032	.098	-.121	.111	**.363**	.215	-.015		
	9. EFT RT	-.182	-.233	.188	**.292**	-.192	.028	.221	-.069	

[42] All correlational coefficients displayed are Spearman's rho; significant correlations in bold type; *sample sizes ranged from 61 to 66; AQ soc.: Autism-Spectrum Quotient higher order social interaction factor subscale; AQ att.det: Autism-Spectrum Quotient attention to detail factor subscale; EQ: Empathy Quotient; SQ-R: Systemizing Quotient-Revised; Eyes: Mind in Eyes; KDEF: Karolinska Directed Emotional Faces; ART: Accuracy-adjusted Response Time (transformed); EFT: Embedded Figures Task; RT: Response Time.*

Table 7.3: *Results of group comparisons; (a) high versus low-medium autistic traits (social) and (b) high versus low-medium autistic traits (non-social).*[43]

(a)

	ASC Fathers			ASC Mothers		
Measure	**High autistic traits (social) (N = 10)**	**Low-medium autistic traits (social) (N = 50)**	**Group comparison (p value)**	**High autistic traits (social) (N = 10)**	**Low-medium autistic traits (social) (N = 50)**	**Group comparison (p value)**
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
AQ Att.det.	30.2 (5.14)	23.7 (4.49)	$p < 0.001$	26.8 (3.80)	21.0 (6.05)	$p < 0.001$
SQ-R	79.1 (18.04)	60.8 (18.03)	$p < 0.01$	59.7 (19.61)	42.5 (17.89)	$p < 0.01$
EQ	19.4 (7.20)	37.6 (12.80)	$p < 0.001$	27.5 (12.94)	55.9 (10.37)	$p < 0.001$
KDEF ART (msecs)	3094.49 (752.51)	3200.91 (717.53)	n.s.	3045.68 (923.48)	2979.41 (740.48)	n.s.
Eyes accuracy (/36)	25.5 (4.86)	25.4 (4.32)	n.s.	25.8 (4.02)	26.8 (4.00)	n.s.
EFT RT (secs)	18.5 (16.92)	25.8 (15.27)	$p < 0.05$	18.6 (8.64)	28.4 (15.91)	$p < 0.05$
	High autistic traits (social) (N = 10)	**Low-medium autistic traits (social) (N = 54)**	**Group comparison (p value)**	**High autistic traits (social) (N = 10)**	**Low-medium autistic traits (social) (N = 56)**	**Group comparison (p value)**
	Median (min-max)	Median (min-max)		Median (min-max)	Median (min-max)	
KDEF accuracy (/20)	17.2 (15.3-18.1)	17.5 (12.4-19.4)	n.s.	17.1 (15.1-18.4)	18.0 (15.0-19.7)	$p < 0.05$

[43] AQ Att.det; AQ attention to detail factor subscale, SQ-R; Systemizing Quotient-Revised, EQ; Empathy Quotient, KDEF; Karolinska Directed Emotional Faces, Eyes; Mind in Eyes, ART; Accuracy-adjusted Response Time

(b)

	ASC Fathers			ASC Mothers		
Measure	**High autistic traits (non-social) (N = 10)**	**Low-medium autistic traits (non-social) (N = 50)**	**Group comparison (p value)**	**High autistic traits (non-social) (N = 10)**	**Low-medium autistic traits (non-social) (N = 50)**	**Group comparison (p value)**
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
AQ Soc.[44]	104.1 (19.24)	90.8 (17.16)	$p < 0.05$	101.5 (13.87)	74.8 (21.12)	$p < 0.01$
SQ-R	87.7 (22.17)	59.04 (14.56)	$p < 0.001$	63.9 (18.00)	41.7 (17.25)	$p < 0.01$
EQ	27.4 (15.63)	35.9 (13.12)	n.s.	40.0 (12.53)	53.4 (14.7)	$p < 0.001$
KDEF ART (msecs)	2922.71 (769.50)	3235.3 (703.85)	n.s.	3088.14 (751.53)	2970.92 (774.36)	n.s.
Eyes accuracy (/36)	25.6 (3.78)	25.4 (4.51)	n.s.	26.6 (4.35)	26.6 (3.96)	n.s.
EFT RT (secs)	16.9 (16.22)	26.2 (15.22)	$p < 0.05$	23.1 (12.13)	27.5 (15.90)	n.s.
	High autistic traits (social) (N = 10)	**Low-medium autistic traits (social) (N = 54)**	**Group comparison (p value)**	**High autistic traits (social) (N = 10)**	**Low-medium autistic traits (social) (N = 56)**	**Group comparison (p value)**
	Median (min-max)	Median (min-max)		Median (min-max)	Median (min-max)	
KDEF accuracy (/20)	17.6 (15.3-18.4)	17.4 (12.4-19.4)	n.s.	17.6 (15.4-19.7)	17.9 (15.0-19.7)	n.s.

[44] AQ Soc; AQ social interaction factor subscale.

7.4.2 Assessing aggregation of autistic traits and related phenotypes in parents with high scores on the AQ.

Tables 7.3 displays the descriptives and results of statistical comparisons between parents separated into high and medium-low scores on autistic traits in the social and non-social domains; groups were compared on three self-report scales and three performance-based tasks. In Table 7.3a participants were assigned high autistic trait status using the social factor subscale of the AQ ('social interaction'), whilst in Table 7.3b participants were assigned high autistic trait status using the non-social factor subscale of the AQ ('attention to detail'). On the self-report scales measuring autistic traits and related phenotypes, ASC mothers and fathers with high scores on the AQ social interaction subscale reported significantly higher SQ-R and AQ attention to detail scores and significantly lower EQ scores than ASC mothers and fathers without high scores on the AQ social interaction subscale (see Table 7.3 for p values). Thus, parents who score highest on the AQ social interaction subscale, also tend to score high on the SQ-R and AQ attention to detail subscale, and low on the EQ. On the performance-based tasks, there were no significant differences between parents with and without high scores on the AQ social interaction subscale except for response times on the EFT ($p < 0.05$) and accuracy scores on the KDEF in ASC mothers ($p < 0.05$). The former result suggests that autistic traits can aggregate across domains (social/non-social) and across measurement types (self-report/ performance-based task) in both mothers and fathers of autistic probands with high scores on this AQ subscale.

ASC parents with and without high scores score on the AQ attention to detail subscale were also compared on the same measures. On the self-report scales measuring autistic traits and related phenotypes, both ASC fathers and ASC mothers with high AQ attention to detail scores were significantly different from those without high AQ attention to detail scores, with the exception of EQ scores in ASC fathers (see Table 7.3 for p values). On the performance-based tasks, there were no significant differences between ASC fathers and ASC mothers with and without high AQ attention to detail scores with the exception of response time on the EFT in ASC fathers ($p < 0.05$). Thus, parents scoring highest on the attention to detail subscale of the AQ scored significantly higher on the SQ-R/ AQ social interaction factor subscale and significantly lower on the EQ (in mothers) but not significantly better or worse on the performance-based cognitive measures, with the exception of response times on the EFT in ASC fathers. These results provide modest evidence to suggest that selected autistic traits and related phenotype (high systemising/ detail-focused cognitive style) aggregate within the non-social domain in ASC fathers.

7.5 Discussion

This study aimed to investigate the relationships between social and non-social autistic traits and related cognitive phenotypes in the first-degree genetic relatives of children/ adolescents with ASC. By doing so, it explores a modified version of the hypothesis proposed by Happé and Ronald (2008) that the DSM-IV defined 'triad of impairments' is fractionable; here the relationships between an autism dyad of social and non-social characteristics was examined, which is more consistent with the latest revision of DSM-5

(APA, 2012), driven by research and diagnostic practice suggesting that the communication and social interaction 'domains' are not reliably distinguishable (Lord and Jones, 2012). Results provided partial support for this hypothesis, with strongest evidence for aggregation of selected non-social traits with related phenotypes (high systemising/ a detail-focused cognitive style) in the fathers of children with ASC. A summary of these results are provided below:

Firstly, in ASC mothers, autistic traits and related cognitive phenotypes aggregated across the social and non-social domains when the measurement type was self-report questionnaire. In ASC fathers the evidence for aggregation across the social and non-social domains was more equivocal, with the lack of a significant association between self-rated empathy and systemising. When the measurement type was a performance-based task, there was some evidence that social cognitive difficulties aggregated separately from a non-social, detail-focused cognitive style, especially in ASC mothers. In ASC fathers there was some evidence for aggregation of test scores across the social and non-social domains; fathers who performed more accurately on a test of mental state recognition (the Mind in Eyes test) tended to be more accurate on the test of attention to detail (the EFT) and fathers who were more accurate at identifying basic emotions on the KDEF tended to be more accurate on the attention to detail task (EFT). Strongest support for separate aggregation of social and non-social characteristics were found when studying correlations between self-report scales and performance-based tasks (i.e. across measurement types); in ASC fathers, response times for correct items on the EFT correlated significantly with total scores on the SQ-R, but not with the self-report scales

measuring social characteristics (EQ and AQ social interaction factor subscale). In ASC fathers there were also significant correlations between performance-based measures of social cognition and self-report scales measuring social characteristics (e.g. the EQ), and so there was some support for aggregation of social traits and related cognitive phenotype across measurement types. Support for separate aggregation of social and non-social characteristics amongst ASC mothers across measurement type was less strong, with only a single significant correlation between a performance-based measure and a self-report measure.

As well as investigating relationships between autistic traits and related cognitive phenotypes in all mothers and fathers of children with ASC, it was also important to assess whether social/ non-social characteristics aggregated separately in parents with high self-rated autistic traits. Firstly, when high autistic trait status was assigned using the social interaction factor subscale of the AQ and the measurement type was self-report questionnaire, the related phenotypes aggregated across the social and non-social domains of ASC. This was true for both ASC mothers and fathers, as evidenced by significant differences between parents with and without the highest scores on the AQ social interaction subscale for the two other self-report scales (AQ attention to detail factor subscale and SQ-R). Attenuated performance on social cognition measures did not aggregate amongst parents with the highest scores on the AQ social interaction subscale, with the exception of KDEF accuracy scores in ASC mothers. In ASC fathers, those with the highest scores on the AQ social interaction subscale were significantly different from the rest of the sample on EFT response times only, suggesting that fathers who self-report

the highest level of autistic traits in the social domain tend to perform significantly faster on the EFT but are not significantly worse on social cognition tasks.

When group status was assigned using the non-social factor subscale of the AQ and the measurement type was self-report questionnaire, the related phenotypes aggregated across the social and non-social domains, with the exception of EQ scores in ASC fathers with high non-social autistic traits. This was indicated by significant differences between parents with and without high scores on the AQ attention to detail subscale on the AQ social interaction factor subscale and the EQ (the latter in mothers only). There was only one significant difference between groups on the performance-based measures, namely response time to correct items on the EFT in ASC fathers. Thus, results from both measurement types (self-report and performance-based task) again provide some evidence for aggregation of selected non-social characteristics in ASC fathers with high autistic traits in the non-social domain.

Whilst general population samples suggest that the social and non-social behavioural domains of ASC are not strongly associated with each other, these domains co-occur together much more frequently in people diagnosed with ASC, by definition. The results in ASC relatives here suggest a profile somewhere between general population and clinical samples. The social and non-social facets of ASC appear to be significantly associated with each other when they are measured using self-report questionnaires, but less so when measured using performance-based tasks. Therefore the pattern of responses on the self-report questionnaires appears to resemble the clinical profile despite a lack of

evidence for a BAP on these measures in ASC parents reported in chapter four. In contrast, strongest support for within-domain but not across-domain aggregation (i.e. fractionation) was found for non-social characteristics (high self-rated systemising and a detail-focused cognitive style) in ASC fathers with high levels of self-reported autistic traits in the non-social domain. It is recommended that future studies should focus on these participants in particular when investigating the fractionation of social and non-social characteristics of ASC in the parents of autistic probands.

It is also recommended that further studies are needed using larger sample sizes and more powerful statistical techniques (e.g. factor analysis) in order to examine the relationships between the social and non-social aspects of ASC more precisely. One study using the same questionnaire measures as used in the current study (the AQ, EQ and SQ-R) conducted confirmatory factor analysis in a large sample including ASC probands, ASC relatives, and general population controls (Grove et al., under revision). This study found evidence for a social ('empathising') factor and a non-social ('systemising') factor in all three subsamples. Strikingly, the correlations between both factors were significantly stronger in ASC probands and ASC relatives compared to controls, suggesting that both factors aggregate more strongly in people with an increased genetic risk for autism. Grove et al.'s findings may also explain why the present study found relatively stronger aggregation for social and non-social traits in our parent sample, compared to the papers reporting these associations in general population samples (e.g. Hoekstra et al., 2008; Ronald et al., 2005).

Future studies also need to take note of the types of measures used; here, there was stronger evidence for associations between social and non-social domains of ASC when the measures used were self-report questionnaires rather than performance-based tasks. It would be fruitful to explore whether these findings of cross-domain correlations on the self-report questionnaires are influenced by 'halo effects', since ASC parents are likely to know a lot about ASC and as a result may overestimate the presence of autistic traits in themselves; however, if this is the case then one would have expected to find significant group differences between ASC parents and controls on these measures, but this wasn't found (see chapter four). Alternatively, another possible reason for these results is that the self-report questionnaires are broader measures of behaviour compared to performance-based tasks, which examine a specific aspect of human cognition (e.g. emotion perception) in a controlled environment; parents may use compensatory strategies when completing cognitive tasks, which may mean their performance is relatively unaffected but they may nevertheless experience milder difficulties in their day-to-day real-world interactions with others that could be detected using self-report questionnaires. This could mean that significant cross-domain associations are largely restricted to self-report questionnaires rather than performance-based tasks. The results here suggest that more studies are needed investigating possible differences in cognitive strategies used by parents to complete performance-based tasks associated with the social and non-social aspects of ASC (e.g. empathy and systemising-related tasks).

It is acknowledged that the conclusions that can be drawn from these results are limited by the sensitivity of the measures used in the analysis. The self-report scales and

performance-based measures used have all been shown to differentiate individuals with ASC from control groups (described in chapters 2 and 3), but the results from previous chapters suggest that some of the measures are less consistent at detecting the BAP in first-degree relatives, including the AQ and SQ-R (see chapter 4; but see Bishop et al., 2004 and Wheelwright et al., 2010 for positive results). Given the negative results reported in previous chapters, it is unclear whether the results of these phenotypic correlations are representative of the BAP, whilst differences in the sensitivities of these measures may have affected how these measures were associated with each other. Furthermore, whilst these measures were categorised into a dyad of social and non-social domains in line with DSM-V revisions of ASC, further studies are needed that examine the extent to which the measures used in this study are associated with these two clinical domains of behaviour.

Despite these limitations, the results of correlations between self-report scales implies that, in people with an increased risk for ASC, there may be common risk factors that drive the social and non-social domains of ASC; these could involve genetic and/ or environmental influences. Possible genetic influences include rare genetic variants of large effect (e.g. CNV; Sebat et al., 2007; Levy et al., 2011) that may affect the social and non-social aspects of ASC at the same time; these variants can be present in parents which could be passed on to their diagnosed offspring. Conversely, common genetic variants of weak effect may influence the social and non-social domains of ASC separately in the general population (Anney et al., 2010; Chakrabarti et al., 2009). The finding that in some fathers detail-focused cognitive style and high self-rated systemising

aggregate in the relative absence of autistic traits and related phenotype in the social domain, suggests that the aetiological factors behind these characteristics are independent from social autistic characteristics (e.g. reduced empathy) in ASC fathers. Further studies are needed investigating the fractionation of the autism dyad in people with ASC, their relatives and general population controls using large sample sizes and a mixture of measurement types (e.g. self-report and performance-based task). These could examine how the individual profiles of social and non-social autistic traits and related phenotypes differ across groups with different genetic vulnerability to ASC. Such studies could offer very important insights into whether the social and non-social symptoms characterising clinical ASC have distinct or overlapping biological causes.

Chapter Eight

A summary of study findings, limitations and implications for future research and practice.

8.1 Summary of findings

This thesis has aimed to provide a comprehensive overview of the Broader Autism Phenotype (BAP), including its conceptual and empirical aspects. In chapter one, a thorough review of previous research on the BAP at a behavioural, cognitive and neural level has highlighted a number of inconsistent findings. Studies have rarely reported consistently positive results for the presence of autistic traits and related phenotypes in the first-degree relatives of individuals with ASC. In younger, 'at risk' siblings of autistic probands, strongest support has been reported for language delay, problems with joint attention behaviours, as well as other attentional atypicalities such as longer disengagement from a central stimulus to a peripheral one. However, many of these studies into the BAP in infant siblings are severely limited because the children are usually too young to be clinically assessed for having the full clinical phenotype and diagnostic status later in life is not always established and reported. In older ASC relatives (siblings or parents), strongest support for a BAP has been reported for pragmatic language skills, social responsiveness and other aspects of reciprocal social interaction using interviews, observational assessments and questionnaire measures of behaviour. Other areas require further investigation, including repetitive restricted behaviours and interests. When studies have used performance-based tasks to test cognitive processes, ToM/ emotion perception difficulties have been most frequently reported in older ASC relatives, with other areas requiring further study (e.g. divided attention). Since this review suggested that some of the strongest support for a BAP was reported for empathy-related difficulties in ASC siblings and/ or parents, the first

empirical study of this thesis (chapter two) used large sample sizes to further explore empathy and emotion recognition in the parents of children with ASC, adults with ASC and IQ-matched controls. This online study did not find evidence for difficulties in recognising basic facial emotions in the parents of children with ASC but did find evidence for self-rated empathy difficulties in fathers of children with ASC. In adults with ASC, results replicated previous findings of basic emotion recognition and self-rated empathy difficulties as well as indicating sex differences for emotion recognition performance but not for self-rated empathy.

Chapters three to six explored whether the BAP was largely restricted to the first-degree relatives from multiplex families rather than simplex families. As well as aiming to resolve some of the inconsistent finding in the BAP research literature (chapter one), these studies tested predictions derived from the hypothesis that differential genetic mechanisms operate in multiplex and simplex autism. Using improved criteria for classifying families into multiplex and simplex categories (set out in chapter three), the unaffected parents from these two different kinds of families were compared using self-report scales and performance-based tasks measuring autistic traits and related phenotypes (empathy and systemising). Using self-report scales (reported in chapter four), no significant group differences were found between multiplex parents, simplex parents and age and education-matched controls. These measures included a questionnaire measure of autistic traits and two related questionnaires measuring self-rated empathy and systemising. Using performance-based tasks (reported in chapter five), significant group differences were found between multiplex and simplex parents on a test

of more advanced ToM after controlling for verbal intelligence. There were also sex-specific differences among multiplex parents, simplex parents and controls on a test of basic facial emotion recognition, which were restricted to basic, negative emotions (sadness and fear). In both social cognition tests, multiplex parents performed significantly worse than simplex parents or controls but there were no significant differences between simplex parents and controls. No significant differences were found on a visuospatial test measuring attention to detail (the EFT). Finally, examination of the BAP in multiplex versus simplex parents was extended to include exploration of problems relating to other psychiatric conditions (chapter six). Using a self-report measure of sub-clinical traits relating to other psychiatric conditions, responses from parents within multiplex families revealed a significantly greater aggregation of traits relating to ADHD, compared to parents from simplex families, a pattern which did not emerge as a significant clinical problem from a parental interview. Furthermore, parents from multiplex families also reported problems consistent with a significantly greater aggregation of ADHD problems in the male siblings of autistic probands compared to those for siblings from simplex families. In summary, the results of these empirical studies of the BAP in multiplex versus simplex autism parents suggested that ToM, basic emotion recognition difficulties and self-rated sub-clinical traits associated with ADHD could be important components of an underlying genetic liability to ASC present in the unaffected parents of autistic probands from multiplex families. These findings are consistent with the hypothesis of differential genetic mechanisms operating in multiplex and simplex autism, but evidence is restricted to the social cognitive aspects of ASC, namely ToM/ emotion perception difficulties. This corroborates a number of previous

studies that find evidence for a BAP for social-related constructs (Bishop et al., 2004; Losh et al., 2009; Scheeren and Stauder, 2008). Moreover, these findings suggest that multiplex families may have an increased vulnerability for ADHD compared to simplex families, possibly suggesting a shared genetic aetiology between both conditions.

After exploring differences in the expression of the BAP in ASC relatives stratified into multiplex and simplex family groups, one conceptual issue remained about how autistic traits and related phenotypes were related to each other within ASC relatives. This was addressed in chapter seven, by pooling multiplex and simplex autism parents into a single group, and examining whether autistic characteristics associated with the social and non-social domains of ASC symptomatology tend to appear in isolation in ASC parents, as reported in general population studies, or aggregate together, which is more likely to be found in clinical ASC samples. Multiple correlations were conducted between self-report scales and performance-based tasks, and statistical tests compared scores on these measures between ASC parents with and without high social and non-social autistic traits. Social and non-social characteristics tended to aggregate together when the measures were self-report scales but less so when the measures were performance-based tasks. Results suggested strongest support for fractionation of the social and non-social characteristics of ASC in those ASC fathers with high scores on the AQ attention to detail subscale; there was aggregation within the non-social domain where high systemising and a detail-focused cognitive style tended to aggregate together, but there was less aggregation of characteristics across the social and non-social domains.

Drawing together the results of these studies across chapters, there are a small number of conflicting findings that must be resolved. Firstly, it is surprising that a BAP for basic emotion recognition was not found in chapter two given findings of significant differences between multiplex parents and either simplex parents or controls for specific negative basic emotions using the same emotion perception test in chapter five. These discrepant results would suggest that the KDEF is capable of detecting subtle differences in emotion perception ability in ASC relatives providing that those relatives are categorised into multiplex and simplex groups. This distinction was not made in the online study in chapter two so it is possible that the majority of the parents in this sample belonged to simplex families, which could have led to non-significant differences between ASC relatives and controls on this test. These apparently discrepant findings may reflect the substantive cognitive heterogeneity that exists on the autism spectrum and by extension the wider genetic liability to ASC present in some first-degree relatives. This may have been particularly so for the online sample of ASC relatives used in chapter two which had not been sub-grouped into multiplex and simplex categories. Furthermore, whilst the online sample used in chapter two was very large, ASC diagnoses could not be verified and instead relied on parental report. In contrast, the simplex and multiplex autism cohorts used in chapters three to six were well characterised and were largely restricted to high functioning children with ASC, which may have further cut down on the heterogeneity in these families. Despite these improvements to the study reported in chapter five, it is recommended that the performance test used for measuring basic facial emotion recognition in ASC relatives and controls involves emotional stimuli that are of a lower intensity than those used in the KDEF task; making the facial expressions of

emotion more subtle increases the sensitivity of the task and the power to detect more subtle differences between ASC relatives and controls (see chapter two, section 2.5). The second set of discrepant findings found across studies was for self-rated empathy, measured using the EQ. In chapter two, ASC fathers, but not mothers, reported significantly lower empathy than same-sex controls. In contrast, the study in chapter four reported no significant differences in mean EQ scores between multiplex parents, simplex parents and controls in either gender. However, the mean EQ score in multiplex parents was lower than controls and similar to that reported in chapter two, so it is possible that with larger sample sizes a significant difference may have been detected in chapter four. Altogether, these findings suggest that deficits in self-rated empathy, if present, are likely to be subtle, requiring large sample sizes to be detected.

8.2 Advances on previous BAP research

This thesis includes a number of new insights and methodological advances compared to previous research on the BAP. Firstly, chapter one is the first comprehensive review of previous research on the BAP since 1998 (see Bailey et al., 1998). This review of research into the BAP has been updated to take into account a number of methodological advances. These include the development of new psychometric scales that are designed to quantitatively measure the clinical ASC phenotype and the broader ASC phenotype in first-degree relatives. Since 1998 there have also been the first neuroimaging studies into the BAP and a greater range of cognitive tasks used to assess the broader cognitive phenotype of ASC. In chapter two, the first empirical study of the thesis, the BAP was

investigated using larger sample sizes than the majority of previous studies on the BAP, with the aim of increasing power to detect subtle differences in the expression of the BAP. This was also the first study to examine self-rated empathy in the first-degree relatives (parents) of autistic probands. Some of the strongest support for a BAP has currently been found for empathy-related measures, which is why empathy and emotion recognition were selected for further investigation in this study. The empirical studies reported in chapters three to six were the first to investigate the BAP in multiplex versus simplex autism parents using a wide range of self-report scales and performance-based tasks, providing a comprehensive picture of the BAP. Of the small number of previous studies to explore the BAP in multiplex versus simplex relatives, the majority used a single measure only (e.g. the SRS or the BPASS; Bernier et al., 2012; Constantino et al., 2006; De la Marche et al., 2012; Virkud et al., 2009). Here, four self-report scales were used (the AQ, EQ, SQ-R and ASR) as well as three performance-based tasks (the KDEF, Mind in Eyes and EFT). These measured autistic traits, empathy, systemising and sub-clinical traits associated with other psychiatric conditions. None of these measures have been used in previous analyses of multiplex versus simplex autism relatives. Also, both verbal and non-verbal IQ was measured so that samples could be accurately matched where appropriate on IQ, and proband diagnosis in each family was carefully verified using two clinical instruments (the 3Di-short parental interview and the ADOS-G). The criteria used for classifying families into multiplex and simplex groups were stricter than employed in many previous studies: they took into account diagnoses of ASC in the extended family and steps were taken to ensure that the unaffected siblings of probands from provisional simplex families were not under a high risk of warranting a clinical

diagnosis of ASC (thus rendering the status of the family multiplex rather than simplex). Focusing on families containing probands with average cognitive ability can also be considered a strength of this study, since this may further cut down on aetiological heterogeneity within the autism spectrum by leaving out cases with severe intellectual disability (in whom the aetiology may be different, e.g. related to severe obstetric complications, or other neurological problems such as epilepsy (Amiet et al., 2008). Finally, in chapter seven a modification of Happé and Ronald's 'fractionable autism triad' hypothesis (Happé and Ronald, 2008) was scrutinised in ASC relatives for the first time by investigating the relationships between BAP characteristics in ASC parents, both within and across the social and non-social domains of impairment.

8.3 Study limitations

A number of methodological limitations have already been addressed for each empirical study reported in this thesis (see the discussion sections in chapter two and chapters four to seven). In summary, the most significant limitations across studies include the following: i) age differences between groups in the study reported in chapter two and a lack of opportunity to verify diagnoses of ASC in the probands for this online study, ii) the control group used in the studies reported in chapters three to five could not be appropriately matched on non-verbal or verbal IQ (but were matched for age and education), iii) there was an absence of control data for the EFT and the ASR in the studies reported in chapters five and six respectively, iv) the control groups consisted of typical adults, who were not necessarily parents themselves; a control group restricted to

parents of typically developing children may have been a more suitable comparison group, v) there was an absence of a suitable clinical control group in all studies (e.g. the parents of a child with Down Syndrome), vi) the questionnaire measures used in these studies were self-report only due to time restrictions; ideally questionnaires would have combined self and informant-report formats for each participant, and vii) there is a need for larger sample sizes in the study reported in chapter seven so that more powerful statistical designs can be used to investigate the relationships between BAP characteristics associated with the social and non-social domains of impairment characterising clinical ASC.

In addition to the above, there are some further caveats to consider when conducting research into the BAP by studying the unaffected parents of autistic probands. Firstly, there is a potential risk of ascertainment bias when recruiting ASC parents to take part in BAP studies. It is conceivable that the parents who display mild autistic traits are less likely to take part in autism family research studies, since research participation typically involves a high level of face-to-face interaction, which could be too stressful for parents who show behavioural signs consistent with the BAP. Furthermore, in the studies reported in chapters three to seven, it was a requirement for both the mother and the father to participate, so parents who were separated were usually ineligible to take part. It is plausible that parents who display signs of the BAP may be impaired in their ability to sustain long-term relationships and may therefore be less likely to have participated in this project. Consequently, the expression of the BAP may have been underrepresented in the sample used for these empirical chapters.

Secondly, without full, in-depth diagnostic assessments of the parents, it is not possible to completely rule out that one or more ASC parents may have warranted a clinical diagnosis of ASC themselves. None of the parents included in our study had a clinical diagnosis of ASC, but given that autism awareness has increased greatly only over the last few decades, autism symptoms in the parental generation may be more likely to have been overlooked compared to those in the offspring's generation. In addition, there may also be another type of bias with regards to the completion of the self-report scales; ASC parents may be very familiar with the profile of traits involved in an ASC diagnosis as well as its heritability, so this could lead some to exaggerate the presence of autistic traits in themselves. Whilst we can't completely exclude that other parents warranted a full clinical diagnosis or were over-estimating the presence of autistic traits in themselves, the lack of significant differences between multiplex and simplex parents and controls on the Autism Spectrum Quotient (a self-report measure of autistic traits) suggest that this is not a major concern in the parent sample used for chapters three to seven.

8.4 Verifying clinical ASC diagnoses for research

The results of the proband diagnosis verification procedure (see chapter three) used for studies reported in chapters four to seven offer insights into the complex clinical picture of children and adolescents with an autism spectrum diagnosis, and raise questions about how to verify clinical diagnoses of ASC for research studies. Ideally, probands would have met full clinical criteria for both the 3Di developmental, diagnostic and dimensional interview (short version) and the ADOS-G, but only 43% of probands did so. A relatively

high number of probands did not meet clinical criteria on either instrument (9%) and so their families were excluded from the sample. As a minimum requirement, probands had to meet criteria on one of these clinical instruments and there had to be converging evidence for clinical impairment on both measures in at least one domain of impairment (reciprocal social interaction or communication). If they did not then these cases were reviewed by a senior autism researcher and a consensus reached on whether to include or exclude the proband (and their parents) from the sample. Thus, great care was taken to employ a systematic approach to verifying diagnoses of ASC that involved both clinical instruments. These studies focused on individuals with HFA or Asperger Syndrome, to try and further restrict aetiological heterogeneity in the study samples. Presentation of clinical symptomatology in these individuals may be difficult to detect using clinical instruments such as the ADOS-G, and so diagnosing these individuals is not always a straightforward process. Results from the proband diagnosis verification process raise important questions about how to verify ASC diagnoses in research studies, especially as there is currently no single 'gold standard' procedure by which to do so. The results here suggest that a number of children and adolescents are being diagnosed by clinicians without necessarily meeting clinical criteria on both observational assessment and parental interview about the proband's developmental history. As described in chapter three, the results of the research diagnoses also suggest that there were strong disparities between the diagnostic label given to the proband by clinicians, as reported by the parents, and the research diagnostic label indicated by the 3Di parental interview. This mirrors findings reported by Lord et al. (2012), which suggested a great variety in subtype diagnoses of ASC depending on study location, even when the same diagnostic

procedures were used. Altogether, these findings suggest that whilst the standards of rigour in verifying diagnoses for research purposes are high, there are nonetheless discrepancies among researchers. In short, the use of different diagnostic verification procedures and criteria in different research studies (or not using any at all) may affect the final results, by influencing who is included or excluded from the sample.

8.5 Theoretical implications of BAP studies and future directions

The results of decades of twin studies and genetic research into ASC strongly suggest that genetic factors play a significant role in the aetiology of ASC (see Ronald and Hoekstra, 2011 for a review). As a consequence, family studies into the BAP have strong implications for autism genetic research. The family studies conducted in this thesis are relevant to autism genetic research in at least three ways: i) by identifying more refined phenotypes that are likely to be under stronger genetic influence than the clinical phenotype, or to index a liability to the condition (endophenotypes) , ii) by attempting to cut down on aetiological heterogeneity by stratifying samples into 'simplex' and 'multiplex' groups, and iii) by improving our understanding of the inter-relations between the social and non-social domains of impairments characterising clinical ASC and by implication their biological causes. These three points are discussed in greater detail below:

8.5.1 Identifying cognitive endophenotypes for ASC.

In psychiatry, an endophenotype is a measurable and heritable characteristic associated with a condition that is more proximal to the genotype than the clinical phenotype (Gottesman and Gould, 2003). If a component of the clinical phenotype is to show strong potential as a useful endophenotype for a psychiatric condition, then it should be present in the unaffected relatives of autistic probands at a higher rate than in the general population (Gottesman and Gould, 2003). This would be the implication if one found statistically significant differences between unaffected relatives and controls for the trait/ characteristic being assessed. In chapter one, a review of the research literature on the BAP implicated a number of traits/characteristics as potential endophenotypes for ASC including pragmatic difficulties, language delay, poorer social skills, reduced social responsiveness and poorer performance on emotion recognition/ ToM / divided attention/ social orienting tasks. Subsequent empirical studies reported in chapters two to six have provided results suggesting that some of these candidate endophenotypes are valid. Firstly, the results of empirical studies reported in chapter five suggest support for attenuated recognition of basic negative emotions as an endophenotype. However, the non-significant differences between ASC parents and controls on the KDEF reported in chapter two calls for a degree of caution in concluding that this test of basic facial emotion recognition is a useful endophenotype. More convincing support was reported for advanced ToM ability as a possible endophenotype, since this was significantly poorer in the parents of multiplex families who would have high genetic loading for ASC compared to parents from simplex families. There was also some support for low self-

rated empathy in ASC fathers versus male controls (chapter two), implicating self-rated empathy as an endophenotype. While these results were not replicated in chapter four, where mean EQ scores, although lower, were not significantly different in ASC parents (multiplex/ simplex) versus controls, this may have been due to relatively modest sample sizes and therefore limited power. In contrast to the findings related to empathy and ToM ability, the results of studies reported in chapters two and four did not provide any support for self-report measures of autistic traits and systemising as endophenotypes, since the scores were not significantly higher in ASC parents (multiplex/ simplex) versus controls. Likewise no differences were found between multiplex and simplex parents on a performance-based measure of systemising/ attention to detail; the EFT.

Finally the results of chapter six appear to validate research that explores cross-syndrome endophenotypes for ASC and ADHD, since ADHD-like difficulties aggregated in the parents of multiplex families when compared to the parents of simplex families. Therefore, the genetic liability to ASC may also confer liability to some symptoms of ADHD, highlighting the possibility of co-morbidity between these two conditions due to some sharing of genetic risk factors. Future studies should use both clinical groups and controls to test a number of potential cross-syndrome endophenotypes including pragmatics, facial emotion recognition, executive functioning, reward in response to social stimuli, sustained attention and sensory functioning (Rommelse et al., 2011).

The results of empirical studies into the BAP in chapters two to five would suggest that future research should focus on self-rated empathy, basic emotion perception and more

advanced ToM ability as endophenotypes for ASC. To be considered as valid endophenotypes for ASC, these measures should also meet a number of additional criteria, outlined by De Geus and Boomsma (2001) and Gottesman and Gould (2003). Firstly, candidate endophenotypes should show evidence of reliability (high test-retest reliability). Secondly, they should show evidence of heritability (genetic influences) in twin or adoption studies. Thirdly, they should be associated with the behaviour or psychopathology of interest. Fourthly, the association between the endophenotype and behaviour of interest must be mediated by genetic factors, and finally, the association between the endophenotype and behaviour of interest must be theoretically meaningful (De Geus and Boomsma, 2001).

Do the measures implicated as endophenotypes in chapters two to five meet these recommended criteria? Basic emotion recognition, ToM ability and self-rated empathy are here assessed against each recommended criterion:

i) Reliability

As a test of basic facial emotion recognition, the KDEF has demonstrated good test-retest reliability (Goeleven et al., 2008), as has self-rated empathy measured using the EQ (Lawrence et al., 2004). However, the test-retest reliability for the Mind in Eyes task has not been reported. Therefore, there is sufficient evidence to suggest that at least two of these measures meet this first criterion.

ii) Evidence of heritability

There have been no twin studies to date investigating the heritability of basic facial emotion recognition performance, but one twin study measuring ERP components sensitive to the processing of emotional expressions suggests that a substantial proportion of variation in emotion recognition can be attributed to genetic factors (Anokhin et al., 2010). However, more studies investigating the heritability of basic emotion recognition are needed. Evidence for the heritability of more advanced ToM ability is less strong, with twin studies suggesting only modest genetic influences (Hughes et al., 2005; Ronald et al., 2006b). However, these studies have been criticised for not testing children at a developmentally appropriate age for the ToM tests used, with the result that variation in test performance was insufficient to provide evidence of genetic influences. Instead, ToM should have been studied in children during the developmentally sensitive period where this ability is fully acquired (3-4 years of age; Wimmer and Perner, 1983; see Viding et al., 2007). Therefore further twin studies are needed investigating the heritability of ToM skills using appropriate ToM tests and participants at a developmentally-appropriate age. Finally, there are currently no twin studies directly investigating the heritability of empathy using self or other-rated scales, with the exception of one study that found evidence of significant heritability for empathic concern (which is considered to be intact in ASC) but not for perspective-taking (a component of cognitive empathy that is considered to be impaired in ASC) (Davis et al., 1994). Further twin and family studies are therefore needed.

iii) Association with behaviour/psychopathology of interest

A number of studies have demonstrated that people with ASC have basic facial emotion recognition deficits (e.g. Ashwin et al., 2006; Pelphrey et al., 2002; see also chapter two), although results are not always consistent (see Harms et al., 2010 for a review). Furthermore, many studies have shown that people with ASC perform significantly worse than controls on tests of ToM, including the Mind in Eyes task (Baron-Cohen et al., 2001a). Finally, studies have shown that people with ASC self-report significantly lower empathy than controls (Baron-Cohen and Wheelwright, 2004; see also chapter two). Thus, there is strong evidence that all of these suggested endophenotypes meet this criterion.

iv) Genetic correlation

There are only two studies published so far that have investigated whether the relationship between basic emotion recognition ability/ ToM ability/ self-rated empathy and ASC is mediated by common genetic factors. Using a twin sample from the general population, Jones et al. (2009) reported an association between poorer emotion attribution and increased autistic traits, which was mainly explained by common genetic factors. Ronald et al. (2006b) found that autistic traits, particularly communication impairments, predicted ToM performance in twin pairs from the general population, but did not investigate the genetic association between autistic traits and ToM. More studies are therefore needed analysing the genetic correlation between emotion recognition, ToM, empathy and autistic traits. Ideally, these studies would also include clinical samples.

v) Theoretical explanation of a link between autism and endophenotype

There is strong reason to believe that there is a theoretical basis linking emotion perception/ ToM impairments with ASC. Firstly, one of the behavioural domains characterising the latest DSM-5 classifications of ASC is social communication impairments, which include impairments in the use of multiple non-verbal behaviours, a lack of emotional and social reciprocity and problems initiating or sustaining conversations with others. It is intuitively plausible that a number of these core problems are associated with difficulties perceiving/ identifying the thoughts and emotions of others. There is also empirical evidence that supports the link between early ToM ability and later social communication impairments in children with ASC (Tager-Flusberg, 2003); however more studies are needed, including the investigation of the causal relationships between basic emotion recognition problems and social communication impairments. Nevertheless, it can be argued that emotion recognition and ToM problems may be considered as simpler phenotypes that help to explain a number of behavioural symptoms in only a subset of people diagnosed with the condition. Whilst there is strong reason to believe that these cognitive traits are plausible endophenotypes for ASC, it is less clear whether these endophenotypes mediate the causal pathway between risk genes and clinical ASC (the 'mediational' model), or whether the endophenotypes appear alongside the clinical condition sharing genetic risk variants that cause variation in the endophenotype and clinical ASC (the 'liability-index' model) (Kendler and Neale, 2010). Only the former is useful in obtaining phenotypes that are under stronger genetic influence than the clinical phenotype. Future genetically informative longitudinal studies of twins may help to discriminate between these two different endophenotype models; in

contrast to ASC research, a number of such studies have been conducted in ADHD research (see Wood and Neale, 2010 for a review). It would also be informative to investigate whether manipulating the putative endophenotypes, such as via a cognitive treatment, results in a decline in risk for clinical ASC. A reduction in ASC risk is hypothesized as being consistent with the 'mediational' model but not with the 'index-liability' model (Kendler and Neale, 2010).

In summary, it is clear that more studies are needed in order to validate these suggested endophenotypes, using recommended criteria (e.g. DeGeus and Boomsma, 2001; Gottesman and Gould, 2003), particularly twin studies that test endophenotype models and investigate genetic correlations between the endophenotype and autistic traits in clinical samples.

8.5.2 Is stratification of samples into multiplex and simplex groups useful for cutting down on aetiological heterogeneity in ASC?

In over 80% of ASC cases the biological causes are unknown, and currently a given aetiological mechanism can only account for approximately 1-2% of total cases that have a known cause (Abrahams and Geschwind, 2008). As already described, there is strong evidence to suggest that genetic factors play a major role in ASC aetiology. However, the biological causes that have been put forward to explain idiopathic autism have been extremely wide-ranging, including: genetic factors such as de novo and inherited CNV and SNP, epigenetic factors such as DNA methylation and histone modification, maternally derived antibodies, maternal infection, elevated levels of foetal androgens,

heavy metal exposure and folic acid supplementation (Abrahams and Geschwind, 2008; Currenti, 2010; Grafodatskaya et al., 2010). Given the huge aetiological heterogeneity implicated, it seems likely that researchers stand to gain from stratifying their samples in order to cut down on this heterogeneity. In chapters four to six, ASC parent samples were classified into multiplex and simplex groups in order to increase the likelihood that the aetiology of ASC in a subgroup of families (multiplex) is due to inherited genetic factors as opposed de novo mutations that are not inherited from either parent. Significant differences were found between multiplex and simplex parents, suggesting that this distinction is meaningful and useful in research seeking to identify the various genetic risk factors implicated in ASC (see also Bernier et al., 2012; Bölte and Poustka, 2003; Constantino et al., 2006 and Losh et al., 2008 for further studies finding significant differences between multiplex and simplex relatives on measures of autistic traits and related phenotypes). Future genetic and neurobiological studies could focus on quantitative measures of ToM/ emotion perception in both probands and relatives, to increase the power to detect inherited genetic risk factors for ASC and provide further insights into gene-brain/ cognition-behaviour pathways for ASC. Including both autistic probands and first-degree relatives would also increase sample sizes and potentially increase the power to detect genetic or neurobiological differences associated with ASC.

Whilst these results provide important insights into the BAP in multiplex relatives compared to simplex relatives, there are a number of caveats to bear in mind when applying these classification systems, which are described below:

Firstly, for practical reasons it is rarely possible to fully determine whether a family is truly 'simplex'. For example, members of the extended family are rarely assessed extensively to verify that they do not warrant a clinical diagnosis. Instead, researchers usually rely on informants to report on whether members in the extended family have any clinical diagnoses. It is possible that there are family members who may warrant a diagnosis but who have remained undetected. This is especially likely in the older generations (e.g. uncles, grandfathers) who are less likely to have had access to services like those available to the younger generations.

Secondly, it is possible that the parents of simplex families may have had more offspring with ASC if they had decided to have more children. In the studies conducted in this thesis, probands from simplex families had to have at least one unaffected sibling. However, given that the sibling recurrence rate for autism is around 15-20% (Ozonoff et al., 2011), having one unaffected sibling is not a guarantee that the family would not have been multiplex had more children been born into the family. In addition, parents of a child with special needs often choose not to have further children, which reduce the sizes of families participating in these studies.

Thirdly, and a more conceptual caveat: in cases where there are simplex families containing 'unaffected' parents who display signs of the BAP (e.g. ToM difficulties), should these families still be called 'simplex'? One example is a simplex family that participated in the studies reported in chapters four to six, which contained a father who displayed signs of ToM difficulties on the Mind in Eyes task. He described himself as 'a

loner' who worked in the film industry where he called himself 'the autistic director'. Whilst there were not multiple (≥2) clinical cases of ASC in this family, there was evidence for multiple cases of the broader and narrow (clinical) phenotype of ASC, which could be useful to researchers examining the heritable genetic bases of the ASC phenotype. Therefore, in light of these findings, at least two recommendations can be made for future genetic studies of ASC involving the simplex/ multiplex distinction. Firstly, researchers should be clear and transparent about how they classify participants into simplex and/ or multiplex groups. The studies reported in this thesis suggest that it is not always straightforward to make the distinction between simplex and multiplex autism families and so future genetic studies may benefit from the recognition that applying this dichotomous label can be problematic. Secondly, in genetic studies investigating de novo genetic risk factors for ASC, it would be useful to take steps to ensure that parents from simplex families are not included if they display signs of the BAP, such as ToM difficulties. This is because the expression of BAP characteristics suggests that the putative genetic aetiological factors may be inherited rather than arise de novo. If instead the genetic study is investigating common, heritable genetic factors associated with ASC then it is recommended that both autistic probands and family members displaying the BAP are included in the analysis. The results of the studies carried out here suggest that particular attention should be paid to empathy-related measures (e.g. emotion/ mental state perception) and measures assessing symptomatology associated with ADHD.

8.5.3 Do the social and non-social behavioural domains of ASC have independent causes?

Finally, family research into the BAP can make an important contribution towards understanding the biological underpinnings and relationships between the dyad of impairments that define clinical ASC (social interaction/ communication *and* restricted repetitive interests and behaviour, respectively). By exploring whether the BAP appears within or across the social and non-social domains of ASC, one can make an important contribution to understanding whether or not the DSM-5 defined dyad of impairments fractionate (cf. Happé and Ronald, 2008). Results from chapter seven suggested that, amongst ASC parents, social and non-social autistic traits and related phenotypes were related to each other when the measurement type used was self-report questionnaire. These phenotypic correlations indicate that there may be aetiological factors responsible for both the social and non-social domains of ASC. Strongest evidence for fractionation of social and non-social autistic traits and related phenotypes occurred in fathers self-reporting high non-social autistic traits on the AQ, where high systemising and a detail-focused cognitive style aggregated in the absence of difficulties with empathy. Very few studies have directly explored the relationships between the social and non-social domains of ASC in first-degree relatives of autistic probands so much more research is needed in this area. If we want to understand how correlated phenotypes are linked to underlying genetic factors, then a genetically informative design is required, including twin or family studies and molecular genetic studies. Neuroimaging studies would also offer insights into how the relationships between autistic traits and psychological processes are linked to underlying neural substrates. In addition, studies investigating

phenotypic associations would also benefit from larger sample sizes than those reported in chapter seven that enable factor analytic studies to be carried out on ASC relatives, probands and general population controls.

8.6 Avenues for further research on the BAP

In addition to genetic studies, more studies are needed that continue to explore differences in the expression of the BAP in multiplex versus simplex autism families. Further studies are therefore required using a variety of measures of autistic traits and related phenotype, such as interviews, questionnaires (self and informant-report), performance-based tasks and neurophysiological techniques (e.g. ERP, fMRI and DTI). These studies need to use clear and concise criteria for classifying families into multiplex and simplex groups, taking into account diagnoses in the extended family, verifying diagnoses in the proband(s), and verifying that the unaffected siblings or parents in the 'nuclear' family do not warrant a clinical diagnosis. Further family studies are required focusing on empathy-related measures in order to determine whether similar results can be independently replicated in new samples.

Whilst we attempted to obtain a full picture of the BAP, it is acknowledged that there is a substantive range of characteristics implicated in the BAP at a cognitive and behavioural level, which extends beyond those measured here (see chapter one), so it would have been informative to use more measures (e.g. divided attention tasks, executive function tasks, questionnaire or interview measures of language/ pragmatics and sensory profiles).

During the parental interview examining clinical and sub-clinical problems relating to other psychiatric conditions, parents were also asked whether they had experienced problems that were similar to those of their diagnosed child. Despite the lack of significant results on a number of measures reported in chapters four and five, 55% of ASC parents interviewed said they had experienced problems that resembled their diagnosed child, including 58% from multiplex families and 52% from simplex families, with the nature of those problems varying greatly (e.g. sensory sensitivities, compulsive behaviours, restricted interests, social isolation, a lack of social understanding, perfectionism etc.; see Table 8.1). This suggests that it would be of interest to administer measures beyond those used here to investigate other aspects of the ASC phenotype in greater detail in first-degree relatives; in particular, the results from Table 8.1 suggests that future studies should scrutinise sensory abnormalities and restricted interests more heavily in ASC parents. Further family studies will help to establish whether these reports of parent-proband resemblances are genuine and implicate a common cause or whether these reports are epiphenomena, caused, for example, by parents having a heightened awareness of ASC symptomatology and its possible causes as a result of having children with ASC diagnoses.

Table 8.1: *Descriptions of parent-proband resemblances provided by ASC parents during the 3Di parental interview. Parents were asked the question: 'Have you ever had any problems like [proband's name]?* [45]

Descriptions of parent-proband resemblances	**Multiplex parents (N = 64)**		**Simplex parents (N = 60)**	
	N[46]	%[47]	N	%
Collecting and hoarding	3	4.7	0	0
Sensory sensitivities; light, sound, smell, touch and taste	16	25.0	11	18.3
Compulsive behaviours/ rituals	2	3.1	1	1.7
Restricted interests	15	23.4	13	21.7
Strong attention to details	5	7.8	6	10
Concentration difficulties	4	6.3	1	1.7
Pedantic	0	0	1	1.7
Problems forming friendships	4	6.3	1	1.7
Perfectionistic	3	4.7	1	1.7
Conversational disinhibition	1	1.6	0	0
Social isolation	5	7.8	3	5.0
Lack of social understanding	8	12.5	0	0
Problems starting conversations	3	4.7	2	3.3
Solitary play as a child	1	1.6	2	3.3
Eye contact difficulties	4	6.3	1	1.7
Clumsiness	2	3.1	2	3.3
Excellent memory recall	1	1.6	2	3.3
Late speech	2	3.1	1	1.7
Tactless	1	1.6	0	0
Fixations	0	0	1	1.7
Difficulties dividing attention	1	1.6	2	3.3
Preference for precision and structure	0	0	2	3.3
Emotional control difficulties	1	1.6	0	0
Monotone voice	1	1.6	0	0

[45] In cases where a parent wasn't present to complete this section of the 3Di interview, the other parent answered on behalf of their spouse (N=2).
[46] N = Number of parents reporting parent-proband resemblance.
[47] % = Percentage of sample reporting parent-proband resemblance.

8.7 Practical implications of BAP studies

Lastly, it is important to briefly consider what practical implications may directly follow from family studies into the BAP. Firstly, studies on the BAP have implications for clinical assessments of ASC. One crucial step in the diagnostic assessment is a parental interview where the parents of the individual being assessed are interviewed about their son or daughter's developmental history (e.g. the ADI-R; Lord et al., 1994 and the 3Di; Skuse et al., 2004). Future studies could examine whether the accuracy of parents' responses during this interview is influenced by whether or not they display the BAP. Secondly, if relatives are displaying milder autism-related difficulties that are indicative of the BAP, then it is possible that they may also need some support to improve their relationships with peers and other members of the family. BAP studies will help inform practitioners about the nature of this support. In addition, the results from the study reported in chapter six also suggest that clinicians should be aware of a number of other possible psychiatric problems present in the parents of children with ASC, particularly ADHD symptoms in multiplex parents, and depression/ avoidant personality in multiplex and simplex parents.

8.8 Family research studies and ASC aetiology; past, present and future

To conclude, it is instructive to reflect upon the theoretical advances based on family and twin research over the past 30-40 years towards understanding the aetiology of ASC, and to contrast these advances with the psychoanalytic theories of autism aetiology that

dominated the 1960s and 70s and are still popular in some countries today. Historically one of the most popular psychoanalytic theories of autism aetiology is the 'refrigerator mother hypothesis', which claims that autism is the result of trauma triggered by uncaring and distant mothers who fail to give their child enough emotional support (Bettelheim, 1967). This hypothesis has now been widely discredited. As outlined in this thesis, the weight of scientific evidence strongly suggests that a subset of relatives (parents or siblings) of individuals with ASC display milder characteristics that are qualitatively similar to clinical ASC, which supports observations made by clinicians (e.g. Eisenberg, 1957) that can be traced back to the writings of Hans Asperger and Leo Kanner in the 1940s (Asperger, translated by Frith, 1991; Kanner, 1943, 1949). However, thanks to a combination of BAP family studies (reviewed in this thesis) and a wealth of twin studies (Ronald & Hoekstra, 2011) crucially we can now persuasively conclude that these milder characteristics found in ASC relatives are not responsible for causing the proband's condition via aberrant care-giving but are a consequence of sharing the same aetiological factors (genetic/ environmental) as the diagnosed individual that alter the normal developmental trajectory of the brain and have cascading effects on cognition and behaviour. It is hoped that this thesis and future work on the BAP will make important contributions towards better understanding the nature of these aetiological factors and their influence on neurodevelopment in ASC.

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Appendices

Appendix 1.1 and 1.2: demographic information for BAP studies

Appendix 1.1: *A summary of demographic information for studies examining the BAP in the infant siblings of autistic probands*[48].

	Category	Candidate Traits	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study))	IQ of 1st-degree relatives (range if > 1 study)
Behavioural level	1. Language and communication	• Semantic-pragmatic language	TD	35 Sibs-ASD; 42 Sibs-TD	9.9 Sibs-BAP; 10.2 Sibs-non-BAP; 9.8 Sibs-TD	111 Sibs-BAP; 107 Sibs-non-BAP; 104 Sibs-TD
		• Language delay	TD	29-1,397 Sibs-ASD; 18-27 Sibs-TD	1.5-9.7 Sibs-ASD; 1.5-4.4 Sibs-TD	105 Sibs-ASD; 103 Sibs-TD. IQ not measured in two studies.
		• Rate of communicating	TD	42 Sibs-ASD; 20 Sibs-TD	1.7 Sibs-ASD; 1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD
	2. Motor development	• Reduced time spent in different postures	TD	21 Sibs-ASD; 18 Sibs-TD	1.5 Sibs-ASD; 1.5 Sibs-TD	Not measured
	3. Social interaction	• Response to name	TD	55-101 Sibs-ASD; 43-46 Sibs-TD	0.5-1.0 Sibs-ASD; 0.5-1.0 Sibs-TD	Not measured
		• Initiation of Joint Attention	TD	8-42 Sibs-ASD; 9-20 Sibs-TD	0.7-1.7 Sibs-ASD; 0.7-1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD. IQ not measured in two studies.
		• Response to Joint Attention	TD	8-46 Sibs-ASD; 9-35 Sibs-TD	07-1.7 Sibs-ASD; 0.7-1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD. IQ not measured in four studies.
		• Joint attention (combined)	TD	42 Sibs-ASD; 20 Sibs-TD	1.7 Sibs-ASD; 1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD
		• Reduced requesting behaviours	TD	8-77 Sibs-ASD; 9-21 Sibs-TD	1.3-3.0 Sibs-ASD; 1.3-3.0 Sibs-TD	Not measured
		• Response to Social Interaction	TD	8 Sibs-ASD; 9 Sibs-TD	1.4 Sibs-ASD; 1.3 Sibs-TD	Not measured
		• Atypical gaze shifting	TD	12-31 Sibs-ASD; 17-25 Sibs-TD	0.5-1.5 Sibs-ASD; 0.5-1.5 Sibs-TD	Not measured

[48] TD: Typically-developing; ASD: Autism Spectrum Disorder; BAP: Broader Autism Phenotype; Sibs: Siblings.

	Category	Candidate Traits	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study))	IQ of 1st-degree relatives (range if > 1 study)
		• Reduced social smiling/ higher rates of 'neutral affect' during FFSF task	TD	12-31 Sibs-ASD; 19-24 Sibs-TD	0.5-1.5 Sibs-ASD; 0.5-1.5 Sibs-TD	Not measured
		• Weaker mother-infant synchrony for infant-led interactions during free play	TD	21 Sibs-ASD; 21 Sibs-TD	1.2 Sibs-ASD; 1.2 Sibs-TD	Not measured
		• Reduced gaze towards caregiver's eyes, relative to mouth	TD	31 Sibs-ASD; 24 Sibs-TD	0.5 Sibs-ASD; 0.5 Sibs-TD	Not measured
		• Reduced symbolic behaviour during free play	TD	17-42 Sibs-ASD; 19-20 Sibs-TD	1.7-2.8 Sibs-ASD; 1.8-3.0 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD. IQ not measured in one study.
		• Fewer distal gestures (e.g. pointing)	TD	42 Sibs-ASD;20 Sibs-TD	1.7 Sibs-ASD; 1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD
		• Imitation difficulties	TD	42 Sibs-ASD; 20 Sibs-TD	1.7 Sibs-ASD; 1.8 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD
		• Reduced functional play behaviour	TD	17 Sibs-ASD; 19 Sibs-TD	2.8 Sibs-ASD; 3.0 Sibs-TD	Not measured
	4. Repetitive, restrictive behaviours and interests	• Higher non-functional repeated behaviours	TD	17 Sibs-ASD; 19 Sibs-TD	2.8 Sibs-ASD; 3.0 Sibs-TD	Not measured
Cognitive level	5. Social cognition	• 'Theory of Mind' understanding	TD	24 Sibs-ASD; 24 Sibs-TD	4.5 Sibs-ASD; 4.5 Sibs-TD	Not measured
	6. Executive function	• Enhanced working memory for non-social targets	TD	25 Sibs-ASD; 30 Sibs-TD	0.5-0.8 Sibs-ASD; 05-0.8 Sibs-TD	Not measured
	7. Visual attention	• Attentional disengagement	TD	17-19 Sibs-ASD; 17-19 Sibs-TD	0.5-0.8 Sibs-ASD; 0.5-0.8 Sibs-TD	Not measured
		• Difficulties automatically orienting to targets/ forming expectations about visual environment	TD	19 Sibs-ASD; 19 Sibs-TD	0.8 Sibs-ASD; 0.8 Sibs-TD	Not measured

	Category	Candidate Traits	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study))	IQ of 1st-degree relatives (range if > 1 study)
		• Face processing: increased attention to mouth, relative to eyes	TD	31 Sibs-ASD; 24 Sibs-TD	0.5 Sibs-ASD; 0.5 Sibs-TD	Not measured
	8. Language ability	• Receptive/ expressive language abilities	TD	28-64 Sibs-ASD; 20-42 Sibs-TD	1.3-7.8 Sibs-ASD; 1.3-7.6 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD. IQ not measured in three studies
	9. Contrast sensitivity	• Luminance contrast sensitivity	TD	13 Sibs-ASD; 26 Sibs-TD	0.5 Sibs-ASC; 0.5 Sibs-TD	Not measured
	10. General Cognitive abilities	• Delays in general cognitive development	TD	21-42 Sibs-ASD; 20-47 Sibs-TD	0.3-7.8 Sibs-ASC; 0.3-7.6 Sibs-TD	99 Sibs-ASD; 101 Sibs-TD. IQ not measured in three studies.

Appendix 1.2: *A summary of demographic information for studies examining the BAP in older relatives*[49].

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
Behavioural level	1. Language and communication	• Pragmatic difficulties	Parent	TD and Clinical (SLI & DS)	30-238 Parents-ASD; 22-187 TD controls; 30 Parents-SLI; 21-75 Parents-DS	40.0-46.4 Parents-ASD; 40.0-49.2 TD controls; 43.4-43.8 Parents-SLI; 44.8 Parents-DS	108.4-117.2 Parents-ASD; 125.7 TD controls; 112.4 Parents-DS
			Siblings	TD and Clinical (DS, MR & DLD)	27-87 Sibs-ASD; 46 TD controls; 23 Sibs-MR; 22 Sibs-DLD; 64 Sibs-DS	9.2-18.9 Sibs-ASD; 12.2 TD controls; 9.8 Sibs-MR; 9.2 Sibs-DLD; 18.5 Sibs-DS	107.4-111.6 Sibs-ASD; 98.1 Sibs-MR; 97.3 Sibs-DLD; 112.5 Sibs-DS
		• Broadly defined communication difficulties	Parents	TD and Clinical (DS)	48-2000 Parents-ASD; 89-1007 TD controls; 60-72 Parents-DS	39.7--44.6 Parents-ASD; 38.9-39.3 TD controls. Ages not reported in a number of studies.	Not reported/ measured
			Siblings	Clinical (DS)	189-332 Sibs-ASD; 64-136 Sibs-DS	Not reported	Not measured
		• Structural language problems	Parents	TD	238 Parents-ASD; 187 TD controls	43.1 Parents-ASD; 48.1 TD controls	Not measured
			Siblings	TD	42 Sibs-ASD; 46 TD controls	12.4-13.0 Sibs-ASD; 12.2 TD controls	FS IQ not reported
		• Reading/ writing/ spelling and articulation problems	Parents	Clinical (DS)	48-285 Parents-ASD; 60-72 Parents-DS;	Not reported	Not measured

[49] TD: Typically-Developing; SLI: Specific Language Impairment; DS: Down's Syndrome; MR: Mental Retardation; DLD: Developmental Language Delay; DD: Developmental Delay; EOS: Early Onset Schizophrenia; OCD: Obsessive-Compulsive Disorder; FS IQ: Full Scale Intelligence Quotient.

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
			Siblings	Clinical (DS, MR & DLD)	189-332 Sibs-ASD; 64-136 Sibs-DS	Not reported	Not measured
	2. Social interaction	• Broadly defined social difficulties	Parents	TD and Clinical (DS)	48-2000 Parents-ASD; 89-1007 TD controls; 60-72 Parents-DS	39.7--44.6 Parents-ASD; 38.9-39.3 TD controls. Ages not reported in a number of studies.	Not reported/ measured
			Siblings	Clinical (DS)	189-332 Sibs-ASD; 64-136 Sibs-DS	Not reported	Not measured
		• Alexithymia	Parents	Clinical (Prader Willi)	439 Parents-ASD; 45 Parents-Prader Willi	39.2 Parents-ASD; 45.0 Parents-Prader Willi	Not measured
		• Reduced quality/ number of social relationships	Parents	TD and Clinical (DS)	48-78 Parents-ASD; 22 TD controls; 60 Parents-DS	39.0-46.4 Parents-ASD; 39.0-48 TD controls	103.7-117.2 Parents-ASD; 125.7 TD controls; 109.8 Parents-DS
		• Reduced social motivation	Parents	Control group not used	690 Parents-ASD	Not reported	113.3-113.9 Parents-ASD
		• Reduced social expressiveness	Parents	Control group not used	690 Parents-ASD	Not reported	113.3-113.9 Parents-ASD
		• Reduced social responsiveness	Siblings	Clinical (other disorders)	149-1397 Sibs-ASD; 45 Sibs-other disorders	10.6-11.9 Sibs-ASD; 11.7 Sibs-other disorders	Not measured
		• Poor social skills	Parents	TD	25-2000 Parents-ASD; 25-1007 TD controls	39.7-43.0 Parents-ASD; 38.9-43.2 TD controls	110.8 Parents-ASD; 112.7 TD. IQ not measured in 4 studies

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
		• Reduced social engagement	Siblings	TD	15 Sibs-ASD; 14 Sibs-TD	12.4 Sibs-ASD; 12.6 Sibs-TD	113.6 Sibs-ASD; 108.4 Sibs-TD
			Parents	TD	238 Parents-ASD; 187 TD controls	43.1 Parents-ASD; 48.1 TD controls	Not measured
	3. Repetitive, restrictive behaviours and interests	• Rigidity	Parents	Clinical (DS)	46-195 Parents-ASD; 55-72 Parents-DS	39.0-40.0 Parents-ASD; 39.0-40.0 Parents-DS. Ages not reported in a number of studies	103.7-103.8 Parents-ASD; 109.8 Parents-DS. IQ not measured in a number of studies
			Siblings	Clinical (DS)	97 Sibs-ASD; 52 Sibs-DS	Not reported	Not measured
		• Circumscribed interests	Parents	TD and Clinical (other handicaps, dyslexia)	35-42 Parents-ASD; 28 Parents-TD; 39 Parents-other handicaps; 27 Parents-dyslexia	43.0-45.0 Parents-ASD; 44.0-49.0 Parents-TD; 44.0-47.0 Parents-dyslexia	109.6-114.5 Parents-ASD; 112.5-115.7 Parents-TD; 110.3-111.5 Parents-dyslexia
		• Broadly defined stereotyped behaviours	Parents	Clinical (DS)	48-285 Parents-ASD; 60-72 Parents-DS	Not reported	Not measured
			Siblings	Clinical (DS)	189 Sibs-ASD; 64 Sibs-DS	Not reported	Not measured
		• Reports of real-life non-social skills and preferences	Parents	TD and Clinical (dyslexia)	42 Parents-ASD; 28 Parents-TD; 27 Parents-dyslexia	43.0-45.0 Parents-ASD; 44.0-49.0 Parents-TD; 44.0-47.0 Parents-dyslexia	109.6-114.5 Parents-ASD; 112.5-115.7 Parents-TD; 110.3-111.5 Parents-dyslexia

		Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
Cognitive level	4. Social cognition	• Theory of Mind ability	Parents	TD	30-83 Parents-ASD; 22-41 Parents-TD	36.9-46.6 Parents-ASD; 36.6-48.0 Parents-TD	97.8-120.3 Parents-ASD; 98.8-125.7 Parents-TD
			Siblings	TD and Clinical (learning disabilities)	18-27 Sibs-ASD; 27 Sibs-TD; 18 Sibs-learning disabilities	11.1 Sibs-ASD; 11.0 Sibs-TD; 12.5 Sibs-learning disabilities	107.4 Sibs-ASD; 104 Sibs-learning disabilities. FS IQ not reported in 1 study.
		• Emotion recognition	Parents	TD and Clinical (schizophrenia)	40-83 Parents-ASD; 32-40 Parents-TD; 35 Parents-schizophrenia	34.6-46.6 Parents-ASD; 39.0-46.7 Parents-TD; 48.9-50.0 Parents-schizophrenia	117.5 Parents-ASD; 121.2 Parents-TD. FS IQ not reported in a number of studies.
			Siblings	TD and Clinical (schizophrenia)	20-40 Sibs-ASD; 40 Sibs-TD; 11 Sibs-schizophrenia	13.5-16.4 Sibs-ASD; 15.1 Sibs-TD; 16.6 Sibs-schizophrenia	113.1 Sibs-ASD; 112.4 Sibs-TD. FS IQ not reported in 1 study
		• Trustworthiness of faces	Parents	TD	83 Parents-ASD; 32 Parents-TD	46.6 Parents-ASD; 46.7 Parents-TD	117.5 Parents-ASD; 121.2 Parents-TD
		• Discerning emotional content of complex social scenes	Parents	TD	83 Parents-ASD; 32 Parents-TD	46.6 Parents-ASD; 46.7 Parents-TD	117.5 Parents-ASD; 121.2 Parents-TD

		Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
		• Differences in face processing strategy	Parents	TD	42 Parents-ASD; 20 Parents-TD	47.0-48.0 Parents-ASD; 44.0 Parents-TD	120-122 Parents-ASD; 117 Parents-TD
		• Face recognition/ memory ability	Parents	TD	33-40 Parents-ASD; 40-64 TD controls	39.4-44.9 Parents-ASD; 39.0-43.3 Parents-TD	Not measured
			Siblings	TD	10 Sibs-ASD; 12 Sibs-TD	13.1 Sibs-ASD; 14.2 Sibs-TD	121.7 Sibs-ASD; 115.8 Sibs-TD
		• Eye gaze processing/ social orienting difficulties	Parents	TD	25 Parents-ASD; 25 Parents-TD	41.4 Parents-ASD; 43.2 Parents-TD	Not measured
	5. Executive Function	• Mental flexibility/ set-shifting, reduced planning ability	Parents	TD and Clinical (MR, EOS)	36-145 Parents-ASD; 28-96 TD controls; 30-40 Parents-MR; 36 Parents-EOS	42.2-44.3 Parents-ASD; 40.9 TD controls; 41.0-43.5 Parents-MR; 48.8 Parents-EOS	107.2 Parents-ASD; 100.6 Parents-MR; 99.7 Parents-EOS. FS IQ scores not reported/ measured in a number of studies.
			Siblings	TD and Clinical (MR, DLD, DD)	13-66 Sibs-ASD; 15-50 TD controls; 28 Sibs-MR; 30 Sibs-DLD; 32 Sibs-DD	9.7-24.5 Sibs-ASD; 10.3-29.7 TD controls; 9.9 Sibs-MR; 9.1 Sibs-DLD; 12.2-13.7 Sibs-DD	99.7-106.7 Sibs-ASD; 101.2 TD controls; 100.3 Sibs-MR; 98.9 Sibs-DLD. FS IQ not reported/ measured in a number of studies

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
		• Ideational Fluency	Parents	TD	145 Parents-ASD; 96 Parents-TD	41.4 Parents-ASD; 40.9 Parents-TD	FS IQ not reported
			Siblings	TD	145 Parents-ASD; 96 Parents-TD	41.4 Parents-ASD; 40.9 Parents-TD	FS IQ not reported
		• Verbal fluency	Parents	TD	145 Parents-ASD; 96 Parents-TD	41.4 Parents-ASD; 40.9 Parents-TD	FS IQ not reported
			Siblings	TD and Clinical (DD, MR & DLD)	14-66 Sibs-ASD; 15-50 TD controls; 32 Sibs-DD; 28 Sibs-MR; 30 Sibs-DLD	10.7-24.5 Sibs-ASD; 10.3-29.7 TD controls; 12.2-13.7 Sibs-DD; 9.9 Sibs-MR; 9.1 Sibs-DLD	99.7-106.7 Sibs-ASD; 101.2 TD controls; 100.3 Sibs-MR; 98.9 Sibs-DLD
		• Design fluency	Parents	TD	145 Parents-ASD; 96 Parents-TD	41.4 Parents-ASD; 40.9 Parents-TD	FS IQ not reported
			Siblings	TD	145 Parents-ASD; 96 Parents-TD	41.4 Parents-ASD; 40.9 Parents-TD	FS IQ not reported
		• Inhibition/ working memory problems (verbal/ spatial)	Parents	TD and Clinical (MR)	11-145 Parents-ASD; 17-96 TD controls; 40 Parents-MR	40.0-42.4 Parents-ASD; 43.1 Parents-TD; 40.9-42.9 Parents-MR	110.8 Parents-ASD; 112.7 Parents-TD
			Siblings	TD and Clinical (DD)	15-66 Sibs-ASD; 14-50 TD controls; 32 Sibs-DD	10.7-14.3 Sibs-ASD; 10.3-13.3 Sibs-TD; 12.2-13.7 Sibs-DD	113.6 Sibs-ASD; 108.4 Sibs-TD. FS IQ not reported in some studies

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
	6. Visual attention, sensory integration and sensorimotor functioning	• local attentional biases/ 'weak central coherence'	Parents	TD and Clinical (dyslexia, EOS, MR & DS)	25-83 Parents-ASD; 25-32 Parents-TD; 27 Parents-dyslexia; 36 Parents-EOS; 30 Parents-MR; 60 Parents-DS	41.4-46.6 Parents-ASD; 42.3.-49.0 Parents-TD; 44.0-47.0 Parents-dyslexia; 48.8 Parents-EOS; 43.5 Parents-MR	107.2-120.3 Parents-ASD; 112.5-122.6 Parents-TD; 110.3-111.5 Parents-dyslexia; 99.7 Parents-EOS; 100.6 Parents-MR
		• Susceptibility to visual illusion	Parents	TD and Clinical (dyslexia)	42 Parents-ASD; 28 Parents-TD; 27 Parents-dyslexia	43-45 Parents-ASD; 44-49 Parents-TD; 44-47 Parents-dyslexia	109.6-114.5 Parents-ASD; 112.5-115.7 Parents-TD; 110.3-111.5 Parents-dyslexia
		• 'Complex' divided, selective attention/ selective inhibition	Siblings	TD	7 Sibs-ASD; 9 TD controls	12.8 Sibs-ASD; 13.9 TD controls	FS IQ not reported
		• Attentional engagement/ disengagement	Parents	TD	25 Parents-ASD; 25 Parents-TD	41.4 Parents-ASD; 43.2 Parents-TD	Not measured
		• Oculomotor abnormalities (e.g. open-loop pursuit gain)	Parents	TD	42 Parents-ASD; 26 Parents-TD	42.4 Parents-ASD; 43.1 Parents-TD	110.8 Parents-ASD; 112.7 Parents-TD
			Siblings	TD	15 Sibs-ASD; 14 Sibs-TD	12.4 Sibs-ASD; 12.6 Sibs-TD	113.6 Sibs-ASD; 108.4 Sibs-TD

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
	7. Language ability	• Phonological Processing	Parents	TD and Clinical (SLI)	22-51 Parents-ASD; 22 TD controls; 35 Parents-SLI	40.1-42.4 Parents-ASD; 38.8 Parents-SLI	109.3-119.8 Parents-ASD; 96.7 Parents-SLI
			Siblings	Clinical (SLI)	50 Sibs-ASD; 36 Sibs-SLI	9.6-10.7 Sibs-ASD; 11.8 Sibs-SLI	114.5-120.7 Sibs-ASD; 93.4 Sibs-SLI
		• Rapid Automatised Naming (RAN)	Parents	TD and Clinical (DS)	48-301 Parents-ASD; 87 Parents-TD; 60 Parents-DS	Ages not reported	FS IQ not reported/ measured
			Siblings	Clinical (MR & DLD)	27-30 Sibs-ASD; 23-28 Sibs-MR; 22-30 Sibs-DLD	9.7 Sibs-ASD; 9.8-9.9 Sibs-MR; 9.1-9.2 Sibs-DLD	106.7-107.4 Sibs-ASD; 98.1-100.3 Sibs-MR; 97.3 - 98.9 Sibs-DLD
		• Receptive and expressive language ability	Parents	Clinical (SLI)	51 Parents-ASD; 35 Parents-SLI	40.1-42.4 Parents-ASD; 38.8 Parents-SLI	109.3-119.8 Parents-ASD; 96.7 Parents-SLI
			Siblings	Clinical (MR, DLD & SLI)	27-50 Sibs-ASD; 23 Sibs-MR; 22 Sibs-DLD; 36 Sibs-SLI	9.6-10.7 Sibs-ASD; 9.8 Sibs-MR; 9.2 Sibs-DLD; 11.8 Sibs-SLI	107.4-120.7 Sibs-ASD; 98.1 Sibs-MR; 97.3 Sibs-DLD; 93.4 Sibs-SLI
		• Reading/ Spelling ability	Parents	TD and Clinical (SLI, DS)	30-122 Parents-ASD; 30 Parents-TD; 35 Parents-SLI; 60 Parents-DS	40.0-43.0 Parents-ASD; 41.5-49.2 Parents-TD; 38.8 Parents-SLI	94.0-119.8 Parents-ASD; 96.7 Parents-SLI

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
			Siblings	Clinical (SLI), MR & DLD)	30-50 Sibs-ASD; 36 Sibs-SLI; 28 Sibs-MR; 30 Sibs-DLD	9.6-10.7 Sibs-ASD; 11.8 Sibs-SLI; 9.9 Sibs-MR; 9.1 Sibs-DLD	106.7-120.7 Sibs-ASD; 93.4 Sibs-SLI; 100.3 Sibs-MR; 98.9 Sibs-DLD
	8. Motion Perception	• Luminance contrast sensitivity/ 'atypical Magnocellular pathway functioning'	Parents	Clinical (DS)	51 Parents-ASD; 52 Parents-DS	44.6 Parents-ASD; 46.4 Parents-DS	105.5 Parents-ASD; 103.5 Parents-DS
			Siblings	TD	12 Sibs-ASD; 39 TD controls	15.1 Sibs-ASD; 15.3 TD controls	108.0 Sibs-ASD; 109.0 TD controls
Psychiatric history	10. Other Psychiatric Conditions	• Depression/ affective disorder	Parents	TD and Clinical (DS, other disorders)	48-195 Parents-ASD; 94 TD controls; 34-72 Parents-DS; 114 Parents-other disorders	35.3-50.0 Parents-ASD; 51.0 Parents-DS; 35.4 Parents-other disorders	FS IQ not reported/ measured
			Siblings	Clinical (DS)	97 Sibs-ASD; 52 Sibs-DS	24.0 Sibs-ASD; 27.0 Sibs-DS	FS IQ not reported/ measured
		• Social phobia	Parents	Clinical (DS)	48 Parents-ASD; 60 Parents-DS	Not reported	FS IQ not reported/ measured
		• Anxiety disorder	Parents	Clinical (DS, other disorders)	48-152 Parents-ASD; 34-60 Parents-DS; 114 Parents-other disorders	35.3 Parents-ASD; 35.4 Parents-other disorders. Ages not reported in a number of studies	FS IQ not reported/ measured
		• OCD	Parents	Clinical (DS, other disorders)	48-195 Parents-ASD; 60-72 Parents-DS; 114 Parents-other disorders	35.3-50.0 Parents-ASD; 51.0 Parents-DS; 35.4 Parents-other disorders	FS IQ not reported/ measured

	Category	Candidate Traits	First-degree relative	Control Group(s) Used	Sample sizes (range if > 1 study)	Mean participant ages in years (range if >1 study)	FS IQ of 1st-degree relatives (range if > 1 study)
		• Motor tics	Parents	Clinical (DS, other disorders)	152-195 Parents-ASD; 72 Parents-DS; 114 Parents-other disorders	35.3-50.0 Parents-ASD; 51.0 Parents-DS; 35.4 Parents-other disorders	FS IQ not reported/ measured
			Siblings	Clinical (DS)	97 Sibs-ASD; 62 Sibs-DS	24.0 Sibs-ASD; 27.0 Sibs-DS	FS IQ not reported/ measured
		• Schizophrenia	Parents	Clinical (DS)	195 Parents-ASD; 72 Parents-DS	50.0 Parents-ASD; 51.0 Parents-DS	FS IQ not reported/ measured
			Siblings	Clinical (DS)	97 Sibs-ASD; 52 Sibs-DS	24.0 Sibs-ASD; 27.0 Sibs-DS	FS IQ not reported/ measured
		• Alcoholism	Parents	Clinical (DS, other disorders)	48-152 Parents-ASD; 60 Parents-DS; 114 Parents-other disorders	35.3 Parents-ASD; 35.4 Parents-other disorders	FS IQ not reported/ measured
Personality Traits	11. Personality Traits	• Rigid; Impulsive; Aloof; Shy; Untactful; Schizoid; reserved; irritable; hypersensitive; neuroticism; undemonstrative; anxious	Parents	TD and Clinical (DS, OCD, EOS & MR)	46-195 Parents-ASD; 64 Parents-TD; 38-72 Parents-DS; 37 Parents-OCD; 34 Parents-EOS; 27 Parents-MR	39.0-47.9 Parents-ASD; 47.6 Parents-TD; 39.0-45.0 Parents-DS; 37 Parents-OCD; 34 Parents-EOS; 27 Parents-MR	103.7-107.8 Parents-ASD; 109.8 Parents-DS; 105.8 Parents-OCD; 99.8 Parents-EOS; 98.0 Parents-MR
			Siblings	Clinical (DS)	97 Sibs-ASD; 52 Sibs-DS	Ages not reported	FS IQ not reported/ measured

Appendix 2: Information sheet for ASC parents.

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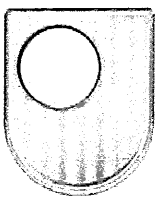

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Information sheet - Parent

Personality characteristics and cognitive abilities in children with an autism spectrum diagnosis and their parents

You and your child are being invited to take part in a study at the Autism Research Centre of the University of Cambridge and the Open University in Milton Keynes. Please consider taking part in this new research project.

What is the purpose of the research project?

The aim of the research is to examine to what extent children with autism resemble their parents on a series of tasks. This study may give us important insights in which characteristics are shared between parents and children and which features are not.

What does the study entail?

We would like to invite both of you as parents and your child with an autism spectrum diagnosis to participate in this project. The study will involve the completion of some questionnaires and assessments on a couple of mental tasks. Prior to the testing day, we will ask you (as parents) to complete some personality questionnaires. Depending on what you prefer, the testing day can either take place at your home, or you can visit our testing rooms in Cambridge or Milton Keynes. The testing session will take about 2.5 to 3 hours in total and you can take breaks during the session as you need to. We will start with a detailed interview that asks about your child's social and communication skills. You will then be given a brief IQ test, and we will ask you to complete three mental tasks. One task involves solving puzzles and two tasks concern the recognition of emotions in other people's faces. Before you do each task, full instructions will be provided and you will also get the chance to practice. Whilst you are completing the tests, we will also ask your child to do an IQ test and to complete the same tasks. Lastly, we will assess the strengths and difficulties of your child during

a standardised assessment. We pay the travel expenses of your family and will in addition present you with a gift voucher of £25 as a thank you for taking part.

What about privacy and confidentiality?
The electronic data we collect will be stored on a secure computer network and any paper-based records will be stored in a secure filing cabinet. In the test results, you will not be identified by name, but by a code number. Your name and contact details will be kept in a separate and secured file. No identifying information will be linked to any of your test data, and all your personal information will be kept strictly confidential. Only members of the research group will have access to the data.

What will happen to the study results?
Results will be presented at conferences and written up in journals. Results will be presented in terms of groups of individuals. All data will be anonymous, without any means of identifying the individuals involved. After completion of the study, your family will be sent a newsletter detailing the results of the study.

What happens if I want to withdraw from the study?
You may withdraw at any stage without explanation and instruct us to destroy your data.

Who has reviewed the study?
The ethics protocol of this study has been reviewed and approved by the Psychology Research Ethics Committee of the University of Cambridge and the Open University Human Participants and Materials Ethics Committee.

I have some more questions about the research. Who can I speak to?
Please contact Edward Sucksmith on 01223 746030 or email es504@medschl.cam.ac.uk

Thank you for reading this.

Appendix 3: A copy of the testing schedule.[50]

Schedule: Edward Sucksmith	**Schedule: Research assistant**	**Approximate Time Taken**
1. Arrival and Introduction: • Take parents and child through the informed consent forms and information sheets • Collect Questionnaires off parents.		
2. 3Di Interview: *rapid autism assessment with extra questions*	2. Cognitive tasks: 2.1 Coloured/ Standard Progressive Matrices (child/ father) 2.2 BPVS (child) 2.3 Mill-Hill Vocab. Scale (father)	60-80 mins
BREAK		10 mins
3. Cognitive tasks (father): 3.1 Mind in Eyes Test 3.2 KDEF Test	3. Cognitive tasks: 3.1 EFT (child) 3.2 Standard Progressive Matrices (mother)	50 mins
4. Cognitive tasks (child): 4.1 Mind in Eyes Test 4.2 KDEF Test	4. Cognitive tasks: 4.1 EFT (father) 4.2 Mill-Hill Vocab. Scale (mother)	20 mins
5. Cognitive tasks (mother): 5.1 Mind in Eyes Test 5.2 KDEF Test	5. Cognitive tasks: 5.1 EFT (mother)	20 mins
6. ADOS-G Child	6. Film ADOS-G if module 2 or finish off tasks with parents.	40 mins
Total Time Taken		220 mins

[50] BPVS: British Picture Vocabulary Scale; EFT: Embedded Figures Task; KDEF: Karolinska Directed Emotional Faces task; ADOS-G: Autism Diagnostic Observational Schedule-Generic

Appendix 4: The Autism-Spectrum Quotient.

		definitely agree	slightly agree	slightly disagree	definitely disagree
1.	I prefer to do things with others rather than on my own.				
2.	I prefer to do things the same way over and over again.				
3.	If I try to imagine something, I find it very easy to create a picture in my mind.				
4.	I frequently get so strongly absorbed in one thing that I lose sight of other things.				
5.	I often notice small sounds when others do not.				
6.	I usually notice car number plates or similar strings of information.				
7.	Other people frequently tell me that what I've said is impolite, even though I think it is polite.				
8.	When I'm reading a story, I can easily imagine what the characters might look like.				
9.	I am fascinated by dates.				
10.	In a social group, I can easily keep track of several different people's conversations.				
11.	I find social situations easy.				
12.	I tend to notice details that others do not.				
13.	I would rather go to a library than a party.				
14.	I find making up stories easy.				
15.	I find myself drawn more strongly to people than to things.				
16.	I tend to have very strong interests which I get upset about if I can't pursue.				
17.	I enjoy social chit-chat.				
18.	When I talk, it isn't always easy for others to get a word in edgeways.				
19.	I am fascinated by numbers.				
20.	When I'm reading a story, I find it difficult to work out the characters' intentions.				
21.	I don't particularly enjoy reading fiction.				
22.	I find it hard to make new friends.				
23.	I notice patterns in things all the time.				
24.	I would rather go to the theatre than a museum.				
25.	It does not upset me if my daily routine is disturbed.				
26.	I frequently find that I don't know how to keep a conversation going.				
27.	I find it easy to "read between the lines" when someone is talking to me.				
28.	I usually concentrate more on the whole picture, rather than the small details.				

		definitely agree	slightly agree	slightly disagree	definitely disagree
29.	I am not very good at remembering phone numbers.				
30.	I don't usually notice small changes in a situation, or a person's appearance.				
31.	I know how to tell if someone listening to me is getting bored.				
32.	I find it easy to do more than one thing at once.				
33.	When I talk on the phone, I'm not sure when it's my turn to speak.				
34.	I enjoy doing things spontaneously.				
35.	I am often the last to understand the point of a joke.				
36.	I find it easy to work out what someone is thinking or feeling just by looking at their face.				
37.	If there is an interruption, I can switch back to what I was doing very quickly.				
38.	I am good at social chit-chat.				
39.	People often tell me that I keep going on and on about the same thing.				
40.	When I was young, I used to enjoy playing games involving pretending with other children.				
41.	I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).				
42.	I find it difficult to imagine what it would be like to be someone else.				
43.	I like to plan any activities I participate in carefully.				
44.	I enjoy social occasions.				
45.	I find it difficult to work out people's intentions.				
46.	New situations make me anxious.				
47.	I enjoy meeting new people.				
48.	I am a good diplomat.				
49.	I am not very good at remembering people's date of birth.				
50.	I find it very easy to play games with children that involve pretending.				

Appendix 5: The Empathy Quotient.

		strongly agree	slightly agree	slightly disagree	strongly disagree
1.	I can easily tell if someone else wants to enter a conversation.				
2.	I find it difficult to explain to others things that I understand easily, when they don't understand it first time.				
3.	I really enjoy caring for other people.				
4.	I find it hard to know what to do in a social situation.				
5.	People often tell me that I went too far in driving my point home in a discussion.				
6.	It doesn't bother me too much if I am late meeting a friend.				
7.	Friendships and relationships are just too difficult, so I tend not to bother with them.				
8.	I often find it difficult to judge if something is rude or polite.				
9.	In a conversation, I tend to focus on my own thoughts rather than on what my listener might be thinking.				
10.	When I was a child, I enjoyed cutting up worms to see what would happen.				
11.	I can pick up quickly if someone says one thing but means another.				
12.	It is hard for me to see why some things upset people so much.				
13.	I find it easy to put myself in somebody else's shoes.				
14.	I am good at predicting how someone will feel.				
15.	I am quick to spot when someone in a group is feeling awkward or uncomfortable.				
16.	If I say something that someone else is offended by, I think that that's their problem, not mine.				
17.	If anyone asked me if I liked their haircut, I would reply truthfully, even if I didn't like it.				
18.	I can't always see why someone should have felt offended by a remark.				
19.	Seeing people cry doesn't really upset me.				
20.	I am very blunt, which some people take to be rudeness, even though this is unintentional.				
21.	I don't tend to find social situations confusing.				
22.	Other people tell me I am good at understanding how they are feeling and what they are thinking.				
23.	When I talk to people, I tend to talk about their experiences rather than my own.				
24.	It upsets me to see an animal in pain.				
25.	I am able to make decisions without being influenced by people's feelings.				
26.	I can easily tell if someone else is interested or bored with what I am saying.				
27.	I get upset if I see people suffering on news programmes.				
28.	Friends usually talk to me about their problems as they say that I am very understanding.				
29.	I can sense if I am intruding, even if the other person doesn't tell me.				
30.	People sometimes tell me that I have gone too far with teasing.				
31.	Other people often say that I am insensitive, though I don't always see why.				

32.	If I see a stranger in a group, I think that it is up to them to make an effort to join in.				
33.	I usually stay emotionally detached when watching a film.				
34.	I can tune into how someone else feels rapidly and intuitively.				
35.	I can easily work out what another person might want to talk about.				
36.	I can tell if someone is masking their true emotion.				
37.	I don't consciously work out the rules of social situations.				
38.	I am good at predicting what someone will do.				
39.	I tend to get emotionally involved with a friend's problems.				
40.	I can usually appreciate the other person's viewpoint, even if I don't agree with it.				

Appendix 6: The Systemising Quotient-Revised.

		strongly agree	slightly agree	slightly disagree	strongly disagree
1.	I find it very easy to use train timetables, even if this involves several connections.				
2.	I like music or book shops because they are clearly organised.				
3.	I would not enjoy organising events e.g. fundraising evenings, fetes, conferences.				
4.	When I read something, I always notice whether it is grammatically correct.				
5.	I find myself categorising people into types (in my own mind).				
6.	I find it difficult to read and understand maps.				
7.	When I look at a mountain, I think about how precisely it was formed.				
8.	I am not interested in the details of exchange rates, interest rates, stocks and shares.				
9.	If I were buying a car, I would want to obtain specific information about its engine capacity.				
10.	I find it difficult to learn how to programme video recorders.				
11.	When I like something I like to collect a lot of different examples of that type of object, so I can see how they differ from each other.				
12.	When I learn a language, I become intrigued by its grammatical rules.				
13.	I like to know how committees are structured in terms of who the different committee members represent or what their functions are.				
14.	If I had a collection (e.g. CDs, coins, stamps), it would be highly organised.				
15.	I find it difficult to understand instruction manuals for putting appliances together.				
16.	When I look at a building, I am curious about the precise way it was constructed.				
17.	I am not interested in understanding how wireless communication works (e.g. mobile phones).				
18.	When travelling by train, I often wonder exactly how the rail networks are coordinated.				
19.	I enjoy looking through catalogues of products to see the details of each product and how it compares to others.				
20.	Whenever I run out of something at home, I always add it to a shopping list				
21.	I know, with reasonable accuracy, how much money has come in and gone out of my bank account this month.				
22.	When I was young I did not enjoy collecting sets of things e.g. stickers, football cards etc.				
23.	I am interested in my family tree and in understanding how everyone is related to each other in the family.				
24.	When I learn about historical events, I do not focus on exact dates.				
25.	I find it easy to grasp exactly how odds work in betting				
26.	I do not enjoy games that involve a high degree of strategy (e.g. chess, Risk, Games Workshop)				

		strongly agree	slightly agree	slightly disagree	strongly disagree
27.	When I learn about a new category I like to go into detail to understand the small differences between different members of that category.				
28.	I do not find it distressing if people who live with me upset my routines				
29.	When I look at an animal, I like to know the precise species it belongs to.				
30.	I can remember large amounts of information about a topic that interests me e.g. flags of the world, airline logos.				
31.	At home, I do not carefully file all important documents e.g. guarantees, insurance policies				
32.	I am fascinated by how machines work.				
33.	When I look at a piece of furniture, I do not notice the details of how it was constructed				
34.	I know very little about the different stages of the legislation process in my country				
35.	I do not tend to watch science documentaries on television or read articles about science and nature.				
36.	If someone stops to ask me the way, I'd be able to give directions to any part of my home town.				
37.	When I look at a painting, I do not usually think about the technique involved in making it.				
38.	I prefer social interactions that are structured around a clear activity, e.g. a hobby.				
39.	I do not always check off receipts etc. against my bank statement				
40.	I am not interested in how the government is organised into different ministries and departments				
41.	I am interested in knowing the path a river takes from its source to the sea.				
42.	I have a large collection e.g. of books, CDs, videos etc				
43.	If there was a problem with the electrical wiring in my home, I'd be able to fix it myself.				
44.	My clothes are not carefully organised into different types in my wardrobe				
45.	I rarely read articles or webpages about new technology.				
46.	I can easily visualise how the motorways in my region link up.				
47.	When an election is being held, I am not interested in the results for each constituency.				
48.	I do not particularly enjoy learning about facts and figures in history.				
49.	I do not tend to remember people's birthdays (in terms of which day and month this falls).				
50	When I am walking in the country, I am curious about how the various kinds of trees differ.				
51.	I find it difficult to understand information the bank sends me on different investment and saving systems.				
52.	If I were buying a camera, I would not look carefully into the quality of the lens.				

		strongly agree	slightly agree	slightly disagree	strongly disagree
53.	If I were buying a computer, I would want to know exact details about its hard drive capacity and processor speed.				
54.	I do not read legal documents very carefully.				
55.	When I get to the checkout at a supermarket I pack different categories of goods into separate bags				
56.	I do not follow any particular system when I'm cleaning at home.				
57.	I do not enjoy in-depth political discussions				
58.	I am not very meticulous when I carry out D.I.Y or home improvements				
59.	I would not enjoy planning a business from scratch to completion.				
60.	If I were buying a stereo, I would want to know about its precise technical features.				
61.	I tend to keep things that other people might throw away, in case they might be useful for something in the future				
62.	I avoid situations which I can not control				
63.	I do not care to know the names of the plants I see				
64.	When I hear the weather forecast, I am not very interested in the meteorological patterns				
65.	It does not bother me if things in the house are not in their proper place.				
66.	In maths, I am intrigued by the rules and patterns governing numbers				
67.	I find it difficult to learn my way around a new city.				
68.	I could list my favourite 10 books, recalling titles and authors' names from memory.				
69.	When I read the newspaper, I am drawn to tables of information, such as football league scores or stock market indices.				
70.	When I'm in a plane, I do not think about the aerodynamics				
71.	I do not keep careful records of my household bills.				
72.	When I have a lot of shopping to do, I like to plan which shops I am going to visit and in what order.				
73.	When I cook, I do not think about exactly how different methods and ingredients contribute to the final product.				
74.	When I listen to a piece of music, I always notice the way it's structured				
75.	I could generate a list of my favourite 10 songs from memory, including the title and the artist's name who performed each song				

Appendix 7: The Adult Self-Report Form

Below is a list of items that describe people. For each item, please circle 0, 1, or 2 to describe yourself over the past 6 months. Please answer all items as well as you can, even if some do not seem to apply to you.

0 = Not True
1 = Somewhat or Sometimes True
2 = Very True or Often True

0	1	2	1. I am too forgetful
0	1	2	2. I make good use of my opportunities
0	1	2	3. I argue a lot
0	1	2	4. I work up to my ability
0	1	2	5. I blame others for my problems
0	1	2	6. I use drugs (other than alcohol and nicotine) for nonmedical purposes (describe): _____
0	1	2	7. I brag
0	1	2	8. I have trouble concentrating or paying attention for long
0	1	2	9. I can't get my mind off certain thoughts (describe) _____
0	1	2	10. I have trouble sitting still
0	1	2	11. I am too dependent on others
0	1	2	12. I feel lonely
0	1	2	13. I feel confused or in a fog
0	1	2	14. I cry a lot
0	1	2	15. I am pretty honest
0	1	2	16. I am mean to others
0	1	2	17. I daydream a lot
0	1	2	18. I deliberately try to hurt or kill myself
0	1	2	19. I try to get a lot of attention
0	1	2	20. I damage or destroy my things
0	1	2	21. I damage or destroy things belonging to others
0	1	2	22. I worry about my future
0	1	2	23. I break rules at work or elsewhere
0	1	2	24. I don't eat as well as I should
0	1	2	25. I don't get along with other people
0	1	2	26. I don't feel guilty after doing something I shouldn't
0	1	2	27. I am jealous of others
0	1	2	28. I get along badly with my family
0	1	2	29. I am afraid of certain animals, situations, or places (describe): _____
0	1	2	30. My relations with the opposite sex are poor
0	1	2	31. I am afraid I might think or do something bad
0	1	2	32. I feel that I have to be perfect
0	1	2	33. I feel that no one loves me
0	1	2	34. I feel that others are out to get me
0	1	2	35. I feel worthless or inferior
0	1	2	36. I accidentally get hurt a lot

0	1	2	37. I get in many fights
0	1	2	38. My relations with neighbors are poor
0	1	2	39. I hang around people who get in trouble
0	1	2	40. I hear sounds or voices that other people think aren't there (describe): _____
0	1	2	41. I am impulsive or act without thinking
0	1	2	42. I would rather be alone than with others
0	1	2	43. I lie or cheat
0	1	2	44. I feel overwhelmed by my responsibilities
0	1	2	45. I am nervous or tense
0	1	2	46. Parts of my body twitch or make nervous movements (describe): _____
0	1	2	47. I lack self-confidence
0	1	2	48. I am not liked by others
0	1	2	49. I can do certain things better than other people
0	1	2	50. I am too fearful or anxious
0	1	2	51. I feel dizzy or lightheaded
0	1	2	52. I feel too guilty
0	1	2	53. I have trouble planning for the future
0	1	2	54. I feel tired without good reason
0	1	2	55. My moods swing between elation and depression
			56. Physical problems **without known medical cause**:
0	1	2	a. Aches or pains (**not** stomach or headaches)
0	1	2	b. Headaches
0	1	2	c. Nausea, feel sick
0	1	2	d. Problems with eyes (not if corrected by glasses) (describe): _____ _____
0	1	2	e. Rashes or other skin problems
0	1	2	f. Stomachaches
0	1	2	g. Vomiting, throwing up
0	1	2	h. Heart pounding or racing
0	1	2	i. Numbness or tingling in body parts
0	1	2	57. I physically attack people
0	1	2	58. I pick my skin or other parts of my body (describe): _____ _____
0	1	2	59. I fail to finish things I should do
0	1	2	60. There is very little that I enjoy
0	1	2	61. My work performance is poor
0	1	2	62. I am poorly coordinated or clumsy
0	1	2	63. I would rather be with older people than with people of my own age
0	1	2	64. I have trouble setting priorities
0	1	2	65. I refuse to talk
0	1	2	66. I repeat certain acts over and over (describe): _____
0	1	2	67. I have trouble making or keeping friends
0	1	2	68. I scream or yell a lot
0	1	2	69. I am secretive or keep things to myself

0	1	2	70. I see things that other people think aren't there (describe): _____
0	1	2	71. I am self-conscious or easily embarrassed
0	1	2	72. I worry about my family
0	1	2	73. I meet my responsibilities to my family
0	1	2	74. I show off or clown
0	1	2	75. I am too shy or timid
0	1	2	76. My behavior is irresponsible
0	1	2	77. I sleep more than most other people during the day and/ or night (describe): _____
0	1	2	78. I have trouble making decisions
0	1	2	79. I have a speech problem (describe): _____ _____
0	1	2	80. I stand up for my rights
0	1	2	81. My behavior is very changeable
0	1	2	82. I steal
0	1	2	83. I am easily bored
0	1	2	84. I do things that other people would think are strange (describe): _____
0	1	2	85. I have thoughts that other people would think are strange (describe): _____
0	1	2	86. I am stubborn, sullen or irritable
0	1	2	87. My moods or feelings change suddenly
0	1	2	88. I enjoy being with people
0	1	2	89. I rush into things without considering the risks
0	1	2	90. I drink too much alcohol or get drunk
0	1	2	91. I think about killing myself
0	1	2	92. I do things that may cause me trouble with the law (describe): _____
0	1	2	93. I talk too much
0	1	2	94. I tease others a lot
0	1	2	95. I have a hot temper
0	1	2	96. I think about sex too much
0	1	2	97. I threaten to hurt people
0	1	2	98. I like to help others
0	1	2	99. I dislike staying in one place for very long
0	1	2	100. I have trouble sleeping (describe): _____ _____
0	1	2	101. I stay away from my job even when I'm not sick and not on vacation
0	1	2	102. I don't have much energy
0	1	2	103. I am unhappy, sad or depressed
0	1	2	104. I am louder than others
0	1	2	105. People think I am disorganised
0	1	2	106. I try to be fair to others
0	1	2	107. I feel that I can't succeed
0	1	2	108. I tend to lose things
0	1	2	109. I like to try new things
0	1	2	110. I wish I were of the opposite sex
0	1	2	111. I keep myself away from getting involved with others

0	**1**	**2**	112. I worry a lot
0	**1**	**2**	113. I worry about my relations with the opposite sex
0	**1**	**2**	114. I fail to pay my debts or meet other financial responsibilities
0	**1**	**2**	115. I feel restless or fidgety
0	**1**	**2**	116. I get upset too easily
0	**1**	**2**	117. I have trouble managing money or credit cards
0	**1**	**2**	118. I am too impatient
0	**1**	**2**	119. I am not good at details
0	**1**	**2**	120. I drive too fast
0	**1**	**2**	121. I tend to be late for appointments
0	**1**	**2**	122. I have trouble keeping a job
0	**1**	**2**	123. I am a happy person
			124. **In the past 6 months**, about how many times per day did you use tobacco (including smokeless tobacco) _____ times per day
			125. **In the past 6 months**, on approximately how many days were you drunk? _____ days
			126. **In the past 6 months**, on how many days did you use drugs for non-medical purposes (including marijuana, cocaine and other drugs, except alcohol and nicotine)? _____

Appendix 8: Supplementary data for chapter three.

1. Highest qualification held by working age adults[1], by gender, age, region and economic activity and, for employees of working age[1], by occupation, 2010 (accessed from the Department of Education's 'Education and Training Statistics for the United Kingdom 2011' in September 2012: **http://www.education.gov.uk***)*

United Kingdom — **Thousands and percentages**

	All working age adults[1] (000s)	NQF level 4 or above[2]	NQF level 3 or above[3]	NQF level 2 or above[4]
Personal and economic characteristics				
By gender				
Males	18,783	36	60	79
Females	17,096	38	57	76
By age				
19-24	5,050	25	61	81
25-29	4,286	45	65	83
30-39	8,026	46	63	81
40-49	9,148	37	55	75
50-64	9,369	33	52	71
By country[5]				
United Kingdom	35,879	37	58	77
England	30,127	38	59	78
Wales	1,673	32	53	74
Scotland	3,051	37	58	77
Northern Ireland	1,028	31	51	71

Labour Force Survey, Quarter 4, 2010[6,7]

[1] Working age adults is defined as males aged 19-64 and females 19-59. These figures include unpaid family workers, those on government employment and training programmes, or those who did not answer, who are excluded from the economic activity analyses below.

[2] **NQF Level 4** includes Higher degrees and other qualifications at Level 5. Also includes First degree, Other degree and sub-degree higher education qualifications such as teaching and nursing certificates, HNC/HNDs, other HE diplomas and other qualifications at Level 4.

[3] **NQF Level 3** includes all qualifications at Level 4 and above in addition to other vocational qualifications such as International Baccalaureate, RSA Advanced Diploma, BTEC Nationals, ONC/ONDs, City and Guilds Advanced Craft or trade apprenticeships and other professional or vocational qualifications at Level 3. Academic qualifications include those with more than one GCE A level or SCE Highers/Scottish Certificates of Sixth Year Studies (CSYS) at Level 3.

[4] **NQF Level 2** includes all qualifications at Level 3 and above in addition to other vocational qualifications such as RSA Diplomas, City and Guilds Craft, BTEC Firsts or trade apprenticeships and other professional or vocational qualifications at Level 2. Academic qualifications include those with one GCE A level, five or more GCSE grades A*-C or equivalent or AS examinations/SCE Highers/CSYS at Level 2.

Appendix 9: Supplementary data for chapter six.

1. Self-reported conditions in parents from multiplex and simplex autism families during the 3Di interview. Includes possible and definite disorders.

Reported condition	Multiplex Parents (N = 64)		Simplex Parents (N = 60)		Fisher exact P value
	N	%	N	%	
Depression	18	28.1	16	26.7	1.0
Anxiety	3	4.7	5	8.3	.48
Bipolar Disorder	1	1.6	1	1.7	1.0
Phobias	0	0	1	1.7	.48
Drug abuse	0	0	1	1.7	.48
Dyslexia	4	6.3	1	1.7	.37
O.C.D	0	0	1	1.7	.48
Personality Disorder	1	1.6	0	0	1.0
P.T.S.D	1	1.6	0	0	1.0
Addictions	1	1.6	0	0	1.0

2. Reported conditions in male siblings of probands from multiplex and simplex autism families during the 3Di interview.

Reported condition	Multiplex brothers (N = 33)		Simplex brothers (N = 17)	
	N	%	N	%
ADHD	10	30.3	0	0
Dyspraxia	3	9.1	0	0
Dyslexia	3	9.1	0	0
Depression	1	3.0	0	0
Pathological Demand Avoidance	1	3.0	0	0
Epilepsy	2	6.1	0	0
Tourette's Syndrome	1	3.0	0	0
PTSD	0	0	0	0
Hyperkinetic conduct disorder	1	3.0	0	0
Conduct Defiance Disorder	1	3.0	0	0

3. Reported conditions in female siblings of probands from multiplex and simplex autism families during the 3Di interview.

Reported condition	Multiplex sisters (N = 14)		Simplex sisters (N = 22)	
	N	%	N	%
ADHD	0	0	1	4.5
Dyspraxia	1	7.1	0	0
Dyslexia	1	7.1	2	0
Depression	2	14.3	0	0
Pathological Demand Avoidance	1	7.1	0	0
Attachment Disorder	0	0	1	4.5
PTSD	0	0	1	4.5
Hypermobility	1	7.1	0	0
Synesthesia	0	0	1	4.5
Phobias	2	14.3	0	0