Redox biology and autism

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Abstract

**Background:** Evidence suggests that oxidative stress may be related to the aetiology of autism. This is supported by studies showing deficiencies in glutathione and other antioxidants, mitochondrial dysfunction and genetic links between autism and abnormalities in redox biology. Glutathione, an important cellular antioxidant, is therefore proposed as a potential treatment target in autism. N-acetyl cysteine (NAC), a glutathione precursor, may be an effective method of supplementing glutathione levels, and thus improving behavioural symptoms and functioning, in children with autism.

**Method:** This study was a mixed-methods, double-blind, randomised, placebo-controlled clinical trial of 500 mg daily NAC, in addition to treatment as usual, for 6 months in children with autistic disorder, as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised. The primary outcomes were the core symptoms of autistic disorder: social interaction, as measured by the Social Responsiveness Scale; communication, as measured by the Children’s Communication Checklist – Second Edition; and restricted and repetitive behaviours and interests, as measured by the Repetitive Behavior Scale – Revised. Secondary outcomes were problem behaviours, as measured by the Developmental Behaviour Checklist – Primary Carer Version; adaptive functioning, as measured by the Vineland Adaptive Behavior Scales – Second Edition; and parent and clinician global impression scales. In addition, qualitative analysis of parent/guardian reports and clinicians’ observations was carried out to supplement the main efficacy study.

**Results:** A total of 98 children (79 male, 19 female; age range = 3.1-10.1 years) were enrolled into the study, of whom 48 were randomised to receive NAC and 50 were randomised to receive placebo. The NAC and placebo groups did not differ on any demographic or baseline symptom severity measure. Seventy-one participants (34 from NAC group, 37 from placebo group) completed the 6-month trial. NAC did not differ from placebo on safety and tolerability. There were no differences between the NAC and placebo groups on any primary or secondary outcome measures. In contrast, the qualitative analysis found that NAC was associated with more frequent reports of improved calmness and verbal communication than placebo.
**Conclusions:** This study found that NAC was not effective in improving core symptoms or functioning in children with autism, as assessed by a range of comprehensive quantitative measures. However, this study did demonstrate the potential utility of mixed-methods approaches in autism treatment trials. Overall, this study does not support the widespread use of NAC for autism, although questions remain regarding dosage, and effects on specific symptoms within the broader clinical picture.
Declarations

This is to certify that:

- The thesis comprises only my original work towards the PhD except where indicated in the Preface,
- Due acknowledgement has been made in the text to all other material used, and
- The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: Kristi-Ann Villagonzalo
Preface

This study was proposed and planned by study investigators: Prof. Michael Berk, Assoc. Prof. Seetal Dodd, Dr Olivia Dean, Assoc. Prof. Kylie Gray, and Prof. Bruce Tonge. All investigators provided ongoing research and clinical guidance.

I was responsible for the coordination of the study, including obtaining all relevant ethics and regulatory approvals; and completing all monitoring and reporting requirements. I was also responsible for recruitment of participants; conducting the majority of study visits, including the collection of quantitative and qualitative data; and data entry and management.

During the study period, trial clinician Ms Tanya Vick assisted with recruitment, conducting study visits and data entry.

KG and trial clinician Ms Deborah Sweeney, both experienced child psychologists, completed thorough case reviews to confirm autism diagnosis for all participants prior to enrolment into the study. Following an increase in project funding, DS administered the Autism Diagnostic Observation Schedule – Generic (ADOS) and cognitive assessments at baseline for all participants subsequently enrolled into the trial. DS also assisted with conducting study visits during the latter part of the study.

I conducted the analysis of demographic data, and secondary analyses. Along with all investigators, I planned the main data analysis, which was then led by statistician Dr Mohammadreza Mohebbi.

Finally, parent/guardian reports and clinicians’ observations were transcribed and the qualitative analysis conducted by Honours candidate Michele Craven, under supervision of Dr Linda Byrne. This work was submitted in partial fulfilment of MC’s Honours degree at Deakin University in 2015. All work previously included in MC’s Honours thesis is cited as such in this thesis.
Funding sources

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- 2010-2011: Australian Rotary Health Research Fund, Pilot Project Grant.

Publications

Journal articles


Poster presentations

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My supervisors, Michael, Seetal and Olivia, have been so many things throughout this journey: sources of wisdom, guidance and inspiration; the nagging voices in my ear; and the beacons leading me back to the research that I loved. I would not have made it to submission day without your time, encouragement, and belief in my abilities. Thank you for everything, and for not giving up on me.

Special thanks go to Kylie Gray and Bruce Tonge, for lending your expertise and for your incredibly helpful feedback along the way. I’m honoured to have learned from you.

For your many and varied contributions to the project and this thesis, thank you to Deb Sweeney, Tanya Vick, Mohammadreza Mohebbi, Michele Craven, Paul Muir, Linda Byrne, and anonymous journal reviewers.

To my awesome family, extended family and in-laws: I thank you all for the laughter, support and love. My family is too big to thank individually, but I need to especially thank my parents, who keep me grounded and inspire me; and Trish, the younger, smarter version of me – you always get me.

Although I’m aware of how cheesy this is, I’m doing it anyway: thanks to my schnauzers, Bonnie and Oscar, for bringing joy and hilarity to my life every day.

To Brendan: you have been with me on every step of this journey, my always positive, always amazing, always loving, number one favourite human of all time. Thank you for the millions of small and huge ways in which you have helped me finish this thing, from tech support and graphic design, to drinks duty, to repeatedly telling me I’m awesome, even when I’m actually being boring and whingey. I love you, always.
This thesis is dedicated to our 98 participants and their families, who gave so generously of their time in the sincerest hope of using their experiences to make a difference. My favourite part of this research was meeting you all, learning from you, and hearing your stories. Thank you for making this research possible, and for being the reason we do research.
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List of Abbreviations

ABC Aberrant Behavior Checklist
ADOS Autism Diagnostic Observation Schedule
ASD autism spectrum disorder
BHMT betaine homocysteine methyltransferase
CATs complementary and alternative therapies
CCC-2 Children’s Communication Checklist – Second Edition
CDPP Centre for Developmental Psychiatry and Psychology (Monash University, Clayton)
CGI-I Clinical Global Impressions – Improvement scale
CGI-S Clinical Global Impression – Severity scale
CRF case report form
DBC-P Developmental Behaviour Checklist – Primary Carer Version
DSM-IV-TR Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised
DSM-5 Diagnostic and Statistical Manual of Mental Disorders, 5th edition
DSMB Data Safety Monitoring Board
EIBI early intensive behavioural intervention
FSIQ Full Scale Intelligence Quotient, WPPSI-III/WISC-IV
GCC General Communication Composite, CCC-2
GCL glutamate cysteine ligase
GCLC glutamate cysteine ligase catalytic subunit
GCLM glutamate cysteine ligase modifier subunit
GEE generalised estimating equation
GFCF gluten- and/or casein-free
GSH-Px glutathione peroxidase
GSH reduced glutathione
GSH-R glutathione reductase
GSSG oxidised glutathione (glutathione disulphide)
HREC Human Research Ethics Committee
ITT intention-to-treat
NAC N-acetyl cysteine
PANSS Positive and Negative Syndrome Scale
PDD pervasive developmental disorder
PDD-NOS pervasive developmental disorder-not otherwise specified
PGI-I Parent Global Impression – Improvement scale
PICF Participant Information and Consent Form
PIQ Performance Intelligence Quotient, WPPSI-III
PRI Perceptual Reasoning Index, WISC-IV
RBS-R Repetitive Behavior Scale – Revised
RCT  randomised controlled trial  
ROS  reactive oxygen species  
SAE  serious adverse event  
SAH  S-adenosylhomocysteine  
SAM  S-adenosylmethionine  
SCQ  Social Communication Questionnaire  
SIDC  Social Interaction Deviance Composite, CCC-2  
SOD  superoxide dismutase  
SRS  Social Responsiveness Scale  
SSRI  selective serotonin reuptake inhibitor  
TBPS  Total Behaviour Problem Score, DBC-P  
VCI  Verbal Comprehension Index, WISC-IV  
VIQ  Verbal Intelligence Quotient, WPPSI-III  
WISC-IV  Wechsler Intelligence Scale for Children – Fourth Edition  
WPPSI-III  Wechsler Preschool and Primary Scale of Intelligence – Third Edition  
XO  xanthine oxidase  

Notes regarding terminology and spelling  

1. This study focuses on autistic disorder, as defined by the DSM-IV-TR, which is abbreviated to ‘autism’ throughout this thesis for brevity.  

2. This thesis uses British English spelling, except where copyrighted titles of assessment tools and measures use American English spelling (i.e. ‘Behavior’ instead of ‘Behaviour’ in the ABC, RBS-R and Vineland-II).
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Chapter 1. Introduction

1.1 Background

Autistic disorder (henceforth referred to as ‘autism’) is a pervasive developmental disorder affecting approximately 1 person in 500 worldwide (1). Its core symptoms, as defined by the Diagnostic and Statistical Manual of Mental Disorders (4th edition, revised; *DSM-IV-TR*) are (a) deficits in social interaction, (b) impaired communication skills, and (c) repetitive or stereotyped behaviours or interests (2). The annual cost associated with autism and its related disorders to Australian society has been estimated at between AU$4.5 billion and AU$7.2 billion (3), stemming from increased use of publicly-funded healthcare and social services, as well as unemployment or under-employment of people with autism and their carers.

There are currently no effective, physiologically-based treatments for the core symptoms of autism. The aetiology of autism is unclear, likely including a combination of genetic susceptibility and exposure to environmental insult. There is a mounting body of evidence suggesting that oxidative stress may play a role in the pathophysiology of autism, and that treatments targeting redox functioning may be effective in improving behavioural symptoms of autism (4). Glutathione is an important antioxidant, and glutathione deficiency and dysfunction have been linked to autism (4). It was hypothesised that N-acetyl cysteine (NAC), a bioavailable precursor of glutathione, would be effective in treating behavioural symptoms of autism.

1.2 The current study

Therefore, the aim of this study was to investigate the efficacy of NAC in children with autism, as well as its safety and tolerability, in a 6-month, double-blind, randomised, placebo-controlled trial. The dosage of 500 mg daily was selected based on titration of adult dose used in previous clinical trials (5,6). Outcomes were comprehensive, valid measures of the three core symptom clusters of autism, in addition to adaptive behaviours, problem behaviours, and parent/guardian and clinician global impression. To supplement the primary efficacy study, qualitative analysis of parent/guardian reports and clinicians’ observations were also carried out.
Chapter 2 reviews the existing knowledge on autism, particularly current treatment and theories on its aetiology. Redox biology and oxidative stress are introduced and defined, and the evidence for their role in autism is outlined. This includes findings on glutathione and other antioxidant deficiencies, mitochondrial dysfunction and genetics, leading to the proposal that antioxidant therapy may be effective in autism. Glutathione is highlighted as a possible therapeutic target, and NAC is identified as a potential treatment for autism.

Chapter 3 describes the methods and measures used in this study, which incorporates a relatively novel mixed-methods clinical trial approach. Chapter 4 details the results of the study on the primary and secondary outcome measures, as well as the findings from the qualitative analysis. Finally, Chapter 5 discusses the findings, and their implications for autism treatment research and the role of oxidative stress in autism. The results are compared to those of four other studies that were conducted contemporaneously, and reasons for discrepancies are identified. The strengths and limitations of this study are also discussed.

At the time the current study was conceived, there were no published studies on the efficacy of NAC in autism. Since then, reports of four small randomised, placebo-controlled trials of NAC in children with autism have been published, three of which found significant improvements in irritability following treatment with NAC (7-10). One study also found that NAC improved social cognition, autistic mannerisms and stereotypies (7). The current study remains the largest and longest trial of NAC in children with autism to date, with a 6-month study period, and 98 participants, aged 3-10 years, recruited from the community. If NAC were found to be effective in treating the core symptoms of autism in the current study, this would provide strong evidence for the role of oxidative stress in the aetiology of autism, and contribute to identification of other potential therapeutic targets.

The trial was registered with the Australian & New Zealand Clinical Trials Registry (registration number: ACTRN12610000635066) and the Therapeutic Goods Administration of Australia.
Chapter 2. Literature review

This chapter provides an overview of the evidence for oxidative stress in autistic disorder (‘autism’). First, autism is defined and described in terms of its prevalence, course, burden and current treatment, highlighting the lack of effective, physiologically-based therapies. This is followed by an outline of current theories on the aetiology of autism. Next, redox biology and oxidative stress are outlined and defined, particularly as they relate to the current study, followed by a review of the evidence for oxidative stress in autism. This includes findings on deficiencies in glutathione and other antioxidants, mitochondrial dysfunction and genetic studies. While it is noted that oxidative stress is tightly linked to inflammation amongst other pathways, these other pathways are outside the scope of this thesis. The evidence leads to the proposal that antioxidant therapy may be effective in autism, and glutathione is identified as a potential therapeutic target. The review concludes with the aims and hypotheses of the current study.

2.1 Overview of autism

Pervasive developmental disorders (PDDs) are a range of syndromes comprising some combination of (a) deficits in social interaction, (b) impaired communication skills, and (c) repetitive or stereotyped behaviours or interests. The classic PDD is autistic disorder, which includes all three core symptom clusters, with onset before the age of three years (2). Table 2.1 describes the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised (DSM-IV-TR) criteria for autistic disorder (2).

The other PDDs, as defined in the DSM-IV-TR, include childhood disintegrative disorder, Rett’s disorder, Asperger’s disorder and pervasive developmental disorder-not otherwise specified (PDD-NOS). Childhood disintegrative disorder is characterised by apparently normal development until at least the age of two years, followed by the loss of previously acquired skills in at least two of the above-listed domains, or in motor skills or bowel/bladder control, before the age of 10 years (2). Similarly, children with Rett’s disorder may seem to develop normally up to around five months of age, but then
<table>
<thead>
<tr>
<th>Symptom cluster</th>
<th>Specific symptoms</th>
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<tr>
<td>Impairments in social interaction</td>
<td>Symptoms must have an onset prior to age of three years and include a total of at least six of the following:</td>
</tr>
<tr>
<td>Impairments in communication</td>
<td>Must include at least two of:</td>
</tr>
<tr>
<td></td>
<td>1. Marked impairments in the use of multiple nonverbal behaviours such as eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction</td>
</tr>
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<td></td>
<td>2. Failure to develop peer relationships appropriate to developmental level</td>
</tr>
<tr>
<td></td>
<td>3. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest to other people)</td>
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<td></td>
<td>4. Lack of social or emotional reciprocity (e.g. not actively participating in simple social play or games, preferring solitary activities, or involving others in activities only as tools or &quot;mechanical&quot; aids)</td>
</tr>
<tr>
<td>Repetitive or stereotyped behaviours or interests</td>
<td>Must include at least one of:</td>
</tr>
<tr>
<td></td>
<td>1. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus</td>
</tr>
<tr>
<td></td>
<td>2. Apparently inflexible adherence to specific, non-functional routines or rituals</td>
</tr>
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<td></td>
<td>3. Stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole-body movements)</td>
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Note: Symptoms must not be better accounted for by Rett’s disorder or childhood disintegrative disorder.
regress and show severe psychomotor and language deficits, as well as loss of social engagement and previously acquired motor skills (2).

Asperger’s disorder differs from autistic disorder in that the former is not associated with a delay in language development or cognition, although impairment in social interaction and repetitive or stereotyped behaviours or interests are still present (2). Finally, children presenting with symptoms from some or all of the above clusters, but not meeting criteria for any specific PDD, may be diagnosed with PDD-NOS (2). These latter two PDDs have more frequently been studied alongside autistic disorder due to their defined overlaps in diagnostic criteria, while Rett’s disorder and childhood disintegrative disorder have often been excluded from such research given their differing presentations and/or aetiologies. Autistic disorder, Asperger’s disorder and PDD-NOS are often referred to collectively as autism spectrum disorders (ASDs).

The 5th edition of the DSM, *DSM-5* (11), was published in 2013, during the recruitment phase of the current study. In the *DSM-5*, the diagnostic classifications for PDDs were significantly revised, comprising a single, dimensional umbrella term of autism spectrum disorder (*DSM-5 ASD*), rather than the five distinct PDD diagnoses included in the *DSM-IV-TR*. The social interaction and communication symptom clusters have been merged into one cluster, comprising deficits in: social-emotional reciprocity; non-verbal communication used for social interaction; and developing, maintaining and understanding relationships. Two symptoms in the repetitive or restricted behaviours or interests cluster are now required for diagnosis. Diagnoses can be further specified as being accompanied by intellectual impairment; language impairment; known genetic, medical or environmentally caused condition; and/or catatonia (11). Rett’s disorder is excluded from the *DSM-5* altogether as it has a clear biological basis (12).

As the current study commenced in 2011, it was completed using the original *DSM-IV-TR* diagnostic criteria for autistic disorder for all participants. This review focuses on autistic disorder as defined by the *DSM-IV-TR*, henceforth referred to as ‘autism’. However, the scope of many of the studies reviewed here includes other ASDs, here referring to the term commonly used to describe autistic disorder, Asperger’s disorder and PDD-NOS collectively; this will be indicated in the review where relevant. This is
not to be confused with the umbrella diagnostic classification of DSM-5 ASD, which is not subsequently used in this review.

2.1.1 Prevalence of autism and other ASDs

The current prevalence of autism is estimated at around 15-20 in 10,000, while the prevalence of all ASDs is commonly estimated at around 60-113 in 10,000 (1,13-27). A comprehensive review of epidemiological studies worldwide found few geographical differences in the prevalence of ASDs, though studies have focused largely on Western countries (28). The prevalence of autism in Australia is consistent with global estimates, at approximately 8.5-36 in 10,000 for children under the age of 16 years (29). The other ASDs, Asperger’s syndrome and PDD-NOS, have estimated prevalence rates of 2.5-48.4 in 10,000 and 15-37.1 in 10,000 respectively (13,30,31).

There is a great deal of variation between individual studies: estimates of the prevalence of autism alone range from 0.7 to 72.6 in 10,000 across 40 years of studies (13). The great variation in prevalence estimates may be due to differences in sample size, sample selection and diagnostic methods used, as well as time (13): the estimated prevalence of autism and other ASDs worldwide has increased approximately ten-fold in the past several decades (13,32-35). Reasons for this rise include changes in diagnostic criteria and improvements in diagnostic instruments, as well as increased awareness and recognition of autism, resulting in earlier diagnosis and inclusion of milder cases (13,36-38).

Other trends are apparent in the prevalence of ASDs. Firstly, ASDs are more common in males than females. Estimates of the male-to-female ratio range between 2.5 and 6.5 for autism (14,19,20,39,40), and 1.3 and 8.3 across all ASDs (16,20-25). Baron-Cohen et al. (41) and others have proposed a combination of causes of this high male-to-female ratio, including exposure to excess foetal testosterone, differences in neuroanatomy, and specific genes on both the X and Y chromosomes (42,43).

As well, ASDs seem to be more prevalent in urban populations (44): almost twice the prevalence in rural populations, according to one review (45). There appears to be no
relationship between race and diagnosis of an ASD (16,30,46). However, the evidence is mixed as to whether ASDs are correlated with parental socioeconomic status (SES). Some studies, particularly in the USA, have found that diagnosis of an ASD is associated with higher parental education and living in a higher income neighbourhood (47-49). Possibly, parents of a higher SES are more likely to seek out and have access to diagnostic and other services where these studies are conducted (47,49). This suggestion is supported by studies in Scandinavia, where there is free universal healthcare and/or routine screening of children for developmental issues (50,51). When there is more parity in access to services, the correlation between higher SES and ASD prevalence disappears (50) or is reversed (51).

2.1.2 Presentation and course of autism

Given the growing recognition and diagnosis rates of autism, it is increasingly important to understand how this disorder presents and impacts individuals across their lifespan. From childhood to adulthood, having autism can have a significant impact on many areas of an individual’s life, including their education, career, relationships, and daily functioning.

On average, the diagnosis of autism occurs at around three years of age, though diagnosis of other ASDs, such as Asperger’s syndrome, tends to occur later, around seven years of age (52). For many parents of children with autism, first concerns arise during the child’s infancy and commonly include poor eye contact, lack of response to name, passivity, and difficulty of temperament (53). On the other hand, up to 50% of parents of children with autism report that their child appeared to develop normally until the age of 18-24 months, then lost language and/or social skills they had previously acquired (54,55). Children who experience such regression tend to show greater levels of language and developmental impairment compared to similarly aged children with autism without regression (55,56).

There are a variety of comorbid behaviours or disorders that commonly present with autism. For example, problematic symptoms frequently associated with autism and other ASDs include: delayed or abnormal motor development (57); sleep disorders,
such as insomnia and nightmares (58); sensory issues, such as sensitivity to loud noises or being touched (59); and dietary symptoms, such as extremely fussy diet or problems with digestion (60). While these symptoms are not required for diagnosis, some combination of these are reportedly experienced by up to 90% of children with ASDs (57,61).

Common physical comorbidities with autism include epilepsy, gastrointestinal dysfunction, immune disorders, and metabolic disturbances (62). Up to 40% of children with autism have co-occurring epilepsy (63). Gastrointestinal problems, including constipation, diarrhoea, and food intolerances, are experienced by 24-54% of children with autism, and are linked to evidence of disrupted microbiota (64-67). Immune disorders, such as asthma, allergies, and autoimmune disorders, occur in up to 40% of children with autism (68,69). Finally, metabolic disturbances, including primary mitochondrial disease, are also relatively common, with a prevalence of around 5% in children with autism (25).

Approximately 50-70% of children with ASDs meet criteria for intellectual disability (70), compared to only 1% in the general population (71). Individuals with both an ASD and intellectual disability show higher rates of problem behaviours (72,73) and poorer outcomes in adulthood compared to those with an ASD only (74). Similar patterns are seen in people with co-occurring psychiatric symptoms in addition to an ASD (75). Mood and anxiety disorders are particularly common, with around 40% of children meeting criteria for such a diagnosis in addition to their ASD (76,77). Other common disorders include obsessive-compulsive disorder, phobias, attention deficit/hyperactivity disorder and oppositional defiant disorder (76). As well, behavioural problems are often seen in ASDs, including disruptive and antisocial behaviour, aggression and self-injury (61,78,79).

Parents of children with autism are more likely than parents of typically functioning children to report that their child has difficulty with school (80). As well, children with autism have more unplanned absences from school, and are less involved in sports and community activities than children without autism (80). Within school, children with
autism tend to have fewer reciprocal friendships, and are less well accepted by their peers (81,82).

Longitudinal studies have shown that the general trend is toward improvement in autistic symptoms through adolescence and adulthood (83). However, having autism remains associated with a range of poorer functional outcomes in adulthood (84). Full-time, independent employment is rare (74). Individuals with autism can struggle to form friendships in adolescence and adulthood (85,86). Where these friendships do exist, they tend to be less subjectively close and supportive than friendships of people without autism (87). Formation of romantic relationships is even less common, with several adult studies finding that few or no participants were in committed relationships (85,88,89).

### 2.1.3 Burden of autism

As well as the pervasive impact for individuals affected by autism and other ASDs, the emotional and financial burden can be substantial for their families. The annual cost of raising a child with an ASD has been estimated at up to three times more than raising a child without (90), due to increased use of health services, medications and special education (90,91). This is compounded by the fact that many parents leave work to provide full-time care for their child (92), or work fewer hours than parents of typically developing children (3,93).

At home, family life may need to revolve around an autistic child's routines, with parents in a qualitative study describing their lives as ‘hectic’ and sometimes ‘feeling robbed’ of a more typical life (94). Over time, caring for a family member with autism may affect emotional health and family relationships. A survey of mothers of children with autism found that maternal mental health declined with increased time spent caring for the child (95). Marital relationships and parents’ relationships with other children in the family may also suffer as a result of the strain (96,97).

Siblings of children with autism report less close and nurturing sibling relationships than siblings of children without autism (98). Those with siblings with autism also
report feeling more loneliness and concern about the future, and may experience more problems with peers (99). Parents of children both with and without autism describe their typically developing child as lonelier and having more behaviour problems than do parents of children without autism (99).

Many individuals with autism continue to live with their parents or other relatives well into adulthood (88,89). Some require assistance with daily tasks such as getting dressed and using the toilet (100). Parents of adults with autism report that there are often insufficient adult and family support services in the community (74), and that they may feel marginalised and unable to live full lives as a result of caring for their adult child (100).

The societal burden associated with autism and other ASDs includes direct costs, such as increased use of publicly-funded healthcare and social services, and indirect costs, such as lost productivity from decreased workforce participation by individuals with ASDs and their carers (3). In Australia, the cost of autism and Asperger’s disorder has been estimated at between AU$4.5 billion and AU$7.2 billion per year (3), while internationally, estimated annual cost is around AU$38 billion (approximately US$28 billion) in the USA (101) and around AU$47 billion (approximately £24.4 billion) in the UK (102). Worldwide, the lifetime societal cost for each individual with autism has been estimated at US$3.2 million (103). In each of these studies, the greatest cost to society stemmed from the loss of productivity from unemployment or under-employment of individuals with ASDs and their carers (3,101,102).

### 2.1.4 Current treatments and their efficacy

The variation in autism symptomatology and its pervasive effect on most aspects of an individual’s life mean that there is currently no clear, universally effective therapeutic approach (104). Therefore, treatment for autism may include a combination of pharmacological, behavioural and alternative therapies, for which the evidence base varies in extensiveness and support.
Many novel approaches are touted as potential treatments or even ‘cures’, often based on very little evidence (105). Sandler and Bodfish (106) described autism as being particularly vulnerable to unsubstantiated claims of the efficacy of new treatments. They attribute this to parents’ understandable desire to improve their child’s quality of life; dissatisfaction with current treatment options; and the sometimes fluctuating nature of autism symptoms, which can give parents hope of a possible breakthrough (106). It is therefore common for parents of children with autism to commence new treatments based on limited supporting evidence (104,107), such as anecdotal reports or information found in the general media (e.g. Internet and newspapers), rather than empirical evidence (108-110).

A brief review of the evidence for currently available treatment options in children with autism follows.

2.1.4.1 Pharmacological treatments

Parent surveys suggest that up to 70% of children and adolescents with autism receive pharmacological treatment (107,111,112), usually for behavioural issues such as aggression, hyperactivity, depression and sleep disturbance, associated with autism (113). Medications used include antipsychotics, antidepressants, anticonvulsants, stimulants and mood stabilisers (114). Prevalence of pharmacological intervention use tends to increase with age, severity of autism and accompanying intellectual disability (111,115).

In one survey, between 50% and 80% of parents indicated that their child improved ‘somewhat’ or ‘dramatically’ while being treated pharmacologically; however, up to 20% reported that their child ‘became worse’ (104). As well, there are a number of adverse side effects associated with the use of pharmacological treatments in children with autism, which may include hyperactivity, aggression, suicidal ideation, fatigue, respiratory symptoms, weight gain and associated metabolic disturbances, sleep disturbance, gastrointestinal symptoms, and hypertension (114,116-118). The adverse event profile of these medications is thus cause for concern, and there may be unforeseen effects on development in autism where changes in brain function are
already present. Given the young age of many children being treated pharmacologically, there is a need for thorough investigation of these medications.

There have been relatively few large, double-blind, placebo-controlled trials of pharmacotherapies in children with autism. Risperidone and aripiprazole are atypical antipsychotics which have a solid evidence base for their efficacy in autism, at a meta-analytic level (116,119,120). In several randomised controlled trials (RCTs), risperidone decreased irritability, self-injury, hyperactivity and aggression in children with autism (121-124), while aripiprazole has similarly been shown to decrease irritability and hyperactivity (125,126). Of the core symptoms of autism, repetitive behaviours and stereotypies were decreased in one large trial of risperidone (122), though other studies have failed to replicate this finding (127).

Other atypical antipsychotics have not yet been studied extensively in RCTs (114,128). Results from small controlled trials, open-label trials and case studies suggest that olanzapine and quetiapine may improve global functioning and behavioural problems in some children with autism (129-131), though these findings are not consistent (128).

Evidence supporting the use of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, fluvoxamine and citalopram, in children with autism is limited (132). Fluoxetine reduced repetitive behaviours and anxiety in a small RCT in children with autism (133,134). However, while fluvoxamine has been shown to be effective in reducing repetitive behaviours, aggression and maladaptive behaviour in an RCT in adults with autism (135), its efficacy is questionable in children, limited in several small RCTs and open-label studies to a small subgroup of responders (134,136). In one large RCT, citalopram was found to be ineffective in treating repetitive behaviours in children with autism (118).

Meanwhile, evidence is similarly inconsistent regarding the use of other antidepressants and anticonvulsants. Reports from open-label trials suggest the potential efficacy of several antidepressants, including clomipramine, venlafaxine and buspirone, in improving repetitive behaviours and behavioural problems such as aggression, irritability and hyperactivity (134,137,138). Anticonvulsants have been proposed as a
possible treatment for autism, due to the prevalence of epilepsy and higher prevalence of abnormal electroencephalogram activity in children with autism (139). Several small RCTs have found that divalproex reduced repetitive behaviours in children with autism (140,141), though these findings were not replicated in other studies (142). Other anticonvulsants, including lamotrigine and levetiracetam, have not shown positive effects in RCTs (143,144).

Oxytocin, a brain peptide, has been proposed as a potential treatment in autism, given its important role in social cognition and bonding (145) and evidence for a possible genetic link to autism (refer to Section 2.1.5). It has been investigated in several RCTs in adults and children with autism (146,147). Encouraging findings have been reported, including improvements in eye gaze, cooperation and emotion recognition (145,148), though these results have not been consistently replicated in larger studies (149,150).

Methylphenidate, a stimulant commonly used to treat attention-deficit hyperactivity disorder, has been shown to be effective in reducing hyperactivity in approximately half of children treated in crossover, placebo-controlled trials (151,152), though it is significantly associated with adverse effects such as irritability and sleep difficulties (152). Finally, the evidence is too limited to speculate on the efficacy of other forms of medication, including mood stabilisers such as lithium, opioid antagonists, non-steroidal anti-inflammatory agents such as celecoxib (153), antibiotics such as D-cycloserine and minocycline (154,155), and glutamate agonists and antagonists, even though some have promising early data (114).

2.1.4.2 Developmental and behavioural therapies

The best evidence base thus far for the treatment of autism is for developmentally- and behaviourally-based interventions, which aim to improve cognitive, communicative and social skills, and reduce problem behaviours and autistic symptomatology, using a range of educational, developmental and behavioural approaches (156). They include early intensive behavioural intervention, parent training, and skills-based therapies such as speech therapy and occupational therapy (107). Up to 93% of children with autism access some form of behavioural intervention at some point (107).
Early intensive behavioural intervention (EIBI) includes a number of developmental and psychoeducational packages, such as Lovaas’ UCLA model (157) and the Denver Model (158). Green et al. (159) described EIBI as having the following characteristics: early – commencing prior to school age, typically around the age of three years; intensive – at least 20 hours a week for at least two years; comprehensive and individualised for a child’s strengths and weaknesses; and the use of behaviour analytic processes focused on developing new behavioural repertoires and reducing problem behaviours. The costs of EIBI, in both time and money, can be extensive. In one qualitative study, nearly all parents using home-based EIBI reported difficulties in areas including obtaining funding, finding reliable therapists, impact on family relationships, and dealing with time-consuming administrative tasks (160).

Reviews of controlled trials of EIBI demonstrate its efficacy in improving language, cognitive abilities, adaptive behaviours, and social functioning in children with autism (156,161-163). However, it is not equally effective for all children. For instance, one review of EIBI for preschool children with autism demonstrated that although there is evidence for the efficacy of this treatment approach, the effect seems to be strongest in the first year of therapy and in children with higher pre-treatment IQ (156). A meta-analysis of EIBI in children with autism found that treatment efficacy was highest in younger children and those with higher pre-treatment levels of adaptive behaviour (161). Two other meta-analyses showed that EIBI efficacy was correlated to the duration and intensity (number of hours) of intervention applied (162,163).

2.1.4.3 Complementary and alternative therapies

Complementary and alternative therapies (CATs) used in autism include: holistic therapies such as yoga or massage/touch-based therapies; as-yet unproven biological therapies including nutritional supplementation with vitamins, amino acids and melatonin; dietary restrictions such as gluten- and casein-free diets; and other forms of physiological treatment such as hyperbaric oxygen therapy and chelation (164,165). Importantly, few such interventions have been shown to be effective in an RCT (164). As well, controversy surrounds the use of several complementary and alternative medicines, with serious safety concerns about treatments such as chelation (166).
Despite the lack of supporting evidence, CATs are used by up to 74% of families with autism (167,168). Parents’ reported reasons for electing to use CATs include the high perceived need for treatment in this group, allied with the perceived lack of side effects (167) and the desire to try multiple treatments to give the best chance of success (164). Up to 75% of parents using a range of CATs in children with autism report improvements in their child’s health or behaviour (169). On the other hand, many parents experience problems with CATs, including high costs for some therapies, difficulty conducting the treatment, and occasionally active harm caused by physiological treatments (170).

The most common types of CAT used are restricted diets and vitamin/mineral supplements (111,167). Dietary interventions in autism include gluten-free or casein-free diets, or a combination thereof (GFCF), and are implemented in up to 50% of children with autism at some stage (171). These diets are based on the proposal that an inability to metabolise these substances results in production of excess peptides, which are then absorbed into the bloodstream and into the brain, contributing to autism symptomatology (172,173). Though parents often report behavioural improvements with the use of these diets (167), particularly in children with co-occurring gastrointestinal problems or food allergies (174), the empirical evidence for GFCF diets is limited.

A 2008 Cochrane review on the efficacy of GFCF diets in autism (175) identified only two RCTs (176,177), both of which were small (n=20 and 15, respectively). The review concluded that while both trials suggested an effect of GFCF diets in improving the symptoms of autism, the evidence was too limited to recommend their use as standard treatment. This conclusion has been reiterated by more recent reviews naming numerous methodological and interpretative issues with a range of studies of GFCF diets in children with autism (178-180). A recent, methodologically rigorous dietary challenge trial in children receiving GFCF diets found no effect on core autism symptoms or behavioural problems (181). Meanwhile, disadvantages of GFCF diets include the cost and inconvenience of sourcing allowed foods, and the effect on nutrition in children who may already have self-restricted diets (173).
Common vitamin/mineral supplements used in children with autism include vitamins A, B6, B12, C and D, magnesium, zinc, iron, and omega-3 fish oils (107, 169). Use of such supplements may be necessary in children with self-restricted diets that cause deficiencies of essential vitamins and minerals (182-184). However, there is limited evidence for the efficacy of many supplements in behavioural symptoms of autism, based mainly on case reports and small trials (185-187). One small RCT found significant improvements on the clinician-rated Clinical Global Impressions – Improvement (CGI-I) scale for children receiving vitamin B12 injections, compared to those receiving placebo injections (188), but found no differences on parent-rated symptom measures.

2.1.4.4 Summary

There is a solid evidence base supporting the use of developmental and behavioural interventions in autism, and some pharmacotherapies for associated behavioural issues; meanwhile, a range of other medications and therapies are commonly used in children with autism without sufficient evidence for their efficacy. This is concerning because ineffective treatments may be costly in both time and money, and may even actively cause harm by being needlessly intrusive, through adverse effects that outweigh their benefits, or by diverting resources that could otherwise be used on more effective treatments (105, 108, 168, 189).

While potential treatments for autism are continually being identified, there is a need for adequately powered RCTs of new treatments (4). In particular, there is a need for treatments that target the core symptoms of autism and have a solid aetiological basis.

2.1.5 Aetiology of autism

While there is not yet a single all-encompassing theory on the aetiology of autism, it is generally accepted that there are most likely genetic and environmental factors, interacting to contribute to neurological and biochemical changes, resulting in the behavioural and biological phenotype of autism (190). A brief outline of some of the research in these areas follows.
The first evidence of a genetic underpinning for autism arose from twin and sibling studies showing high rates of autism in siblings of children with autism, with the highest concordance being 60-90% in monozygotic twins (191-193). Family research has indicated that it is unlikely that there is a single gene for autism; rather, it is likely that there is an additive effect of several genes or sets of genes (194,195). Since then, genetic research in autism has become a sizeable and promising research area, though there are still no definitive answers. In a review of the current status of genetic research in autism, Meek et al. (196) point out that the significant heterogeneity in both the phenotype of autism and the underlying genetics makes it difficult to identify genes responsible for specific behaviours in autism: there may in fact be hundreds of genetic variations involved.

Studies have uncovered potential roles for genes including those related to oxytocin, vasopressin and dopamine receptors, along with many others (195-199). Variations of the OXTR and OXT genes, related to oxytocin levels, have been linked with autism in several studies (196,197), as have vasopressin receptor gene AVPR1a and its microsatellites RS1 and RS3 (198,200). Oxytocin and vasopressin are thought to mediate certain social behaviours including social initiation, responsiveness, and motivation via amygdala activation and this may explain the efficacy seen in some behavioural trials (201,202). Meanwhile, dopamine is essential for attention, learning, and reward value, thus playing a key role in social motivation (203), as well as emotional processing (204). Polymorphisms of the dopamine DRD1 and DRD2 genes have been associated with autism in several studies (205,206). Changes in dopamine-, oxytocin- and vasopressin-related genes in autism may partly explain some of the symptoms seen in autism, including social anxiety, withdrawal and impaired eye contact and joint attention (196).

In addition to the growing number of candidate genes that have been identified in autism, a range of environmental factors has also been implicated in its aetiology, accounting for up to 55% of the risk for developing autism in recent twin studies (207). These include exposure to heavy metals, including mercury, cadmium and nickel; exposure to endocrine disrupting chemicals such as phthalates and bisphenol A (208); prenatal use of valproate; and vitamin D deficiency (209-211). Exposure to heavy
metals in early childhood is associated with a 1.5-2 times increased risk for autism diagnosis (211), potentially by causing oxidative damage to DNA and inhibiting DNA repair (209).

It is likely that genetic susceptibility interacts with exposure to environmental toxins to result in the pathology of autism (190,212). For example, a range of genes have been implicated in impaired metabolism of heavy metals in children with autism (213,214). The wide range of candidate genes and potential environmental factors, and countless possible pathways of interaction between them, mean that it has been challenging to identify biological treatment targets for autism. An area of current research interest is redox biology and oxidative stress (4).

2.2 Overview of redox biology

2.2.1 Oxidative stress

Under normal physiological conditions, reactive oxygen species (ROS), including free radicals and peroxides, are produced as a result of aerobic metabolism (215). These compounds serve important physiological functions such as cellular signalling, immune responses and mitosis (215,216). However, they are highly unstable compounds, which at high levels may cause oxidative damage to cellular proteins, lipids, carbohydrates, and nucleic acids (215). Oxidative stress occurs when there is an imbalance in ROS production against the antioxidant capacity of the cell, due either to excessive ROS generation or to deficiencies in antioxidant defences (215).

There are extensive processes within the cell to prevent oxidative damage and repair it when it does occur (217). Particularly, a range of antioxidants, both enzymatic and non-enzymatic, serve to maintain a reducing environment within the cell by preferentially binding or reacting with ROS, thus deactivating these toxic species (217). Non-enzymatic antioxidants include glutathione and vitamins C and E, while examples of antioxidant enzymes are catalase, superoxide dismutase (SOD), and glutathione peroxidase (215).
Excess ROS production may occur as a result of mitochondrial dysfunction. Mitochondria produce high levels of ROS including hydrogen peroxide and superoxide (218), with defence mechanisms (antioxidants) ordinarily in place within the inner membrane of the mitochondria to neutralise the effects of these ROS (219). When the defences are inadequate, oxidative stress may lead to a cycle of damage to mitochondrial DNA and oxidative defence systems, exacerbating the condition of oxidative stress (220). Primary mitochondrial disease is associated with impaired neurodevelopment, including developmental delay or regression, and may be directly related to behavioural symptoms in at least a subset of children with autism (219).

The brain is particularly vulnerable to oxidative stress, due to limited antioxidant defences and high oxygen utilisation, resulting in increased ROS production (216). As well, the biochemical environment of the brain is particularly conducive to oxidation, due to its high lipid content, reducing potential of neurotransmitters, and the presence of redox-catalytic metals (216). Oxidative stress has been implicated in neuropsychiatric and neurological disorders including schizophrenia, bipolar disorder, major depression, and Parkinson’s and Alzheimer’s diseases (215,221). It has been proposed that oxidative stress, along with other factors including genetic and environmental influences, may contribute to the pathophysiology of autism (218,222).

### 2.2.2 Glutathione

Glutathione (γ-glutamyl-cysteinyl-glycine; empirical formula C$_{10}$H$_{17}$N$_3$O$_6$S) is one of the most important and ubiquitous antioxidants in the human body (223). It is a tripeptide consisting of glutamate, cysteine and glycine (224). As an antioxidant, it acts as a free radical scavenger and helps to eliminate toxic products of oxidative reactions. Glutathione is also essential for antioxidant functioning of other agents (224).

Aside from its important antioxidant properties, glutathione has a number of other critical functions within the cell. These include DNA synthesis and repair; protein and prostaglandin synthesis; modulation of glutamatergic, dopaminergic and serotonergic pathways; amino acid transport and enzyme activation (225-227). It is also essential for immune system functioning, as it helps to regulate the activity of inflammatory
cytokines (228) and may also be important in regulating apoptosis (224). Additionally, glutathione is a detoxifier, binding with both organic and inorganic xenobiotics, including heavy metals such as mercury and lead (224,228). Therefore, glutathione deficiency may have a wide range of deleterious effects.

2.2.2.1 Glutathione synthesis

The rate-limiting factor in the synthesis of glutathione is the availability of cysteine, itself an antioxidant (229). Cysteine, an essential amino acid, is supplied to the body through the diet or as a by-product of the methionine cycle (see Figure 2.1). The methionine cycle involves the transformation of methionine into homocysteine via the intermediates S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). SAM is also critical for cellular methyltransferase reactions, including methylation of DNA, RNA, proteins, phospholipids and neurotransmitters, particularly the monoamines (230,231).

In turn, homocysteine may be returned to methionine via transmethylation, catalysed either by methionine synthase in a folate- and vitamin B12-dependent reaction, or by betaine homocysteine methyltransferase (BHMT) in a reaction independent of folate and vitamin B12 levels. Alternatively, homocysteine may be permanently removed from the methionine cycle by the transsulphuration pathway, where it is converted to cystathionine then cysteine (4).

The synthesis of glutathione from cysteine consists of two steps. In the first, cysteine combines with glutamate to form γ-glutamylcysteine in a reaction catalysed by glutamate cysteine ligase (GCL). The enzyme GCL comprises two proteins, the GCL catalytic subunit (GCLC) and the GCL modifier subunit (GCLM): GCLC acts as the catalyst in the enzymatic reaction, while GCLM increases the efficiency of the catalysis (223). In the second step, glutathione is formed from γ-glutamylcysteine and glycine in a reaction catalysed by glutathione synthetase (223). Excess production of glutathione is prevented by a negative feedback loop between glutathione levels and GCL activity (224).
Figure 2.1. Transmethylation and transsulfuration pathways

*Note.* BHMT, betaine homocysteine methyltransferase; CBS, cystathionine beta-synthase; MAT, methionine adenosyltransferase; MS, methionine synthase; MTase, methyltransferase; SAH, S-adenosylhomocysteine; SAHH, SAH hydrolase; SAM, S-adenosylmethionine; THF, tetrahydrofolate; 5-CH$_3$THF, 5-methyltetrahydrofolate.

Source: Villagonzalo et al. (4)
2.2.2.2 Glutathione and oxidative stress

Under normal conditions, the ratio of reduced glutathione (GSH) to the oxidised form of glutathione (GSSG) within the cell is high, almost 50 to 1 (232). Glutathione reductase (GSH-R) continually reduces GSSG to GSH (223), maintaining the intracellular reducing environment (233,234). Cellular demand for GSH increases when oxidative stress occurs (229). Methionine synthase and BHMT activity are therefore inhibited so that homocysteine is preferentially diverted into the transsulphuration pathway, promoting glutathione synthesis (233). However, under conditions of chronic oxidative stress, this results in methionine depletion, ultimately decreasing homocysteine, cysteine and glutathione levels. Meanwhile, a greater proportion of glutathione becomes oxidised (i.e. the ratio of GSH to GSSG decreases) and GSH-R activity increases to compensate (229). Excess GSSG is exported from the cell, resulting in increased plasma levels of GSSG and a net loss of cellular glutathione (235). Therefore, levels of GSH, GSSG, and the resulting ratio are considered reliable markers of oxidative stress.

2.3 Evidence of oxidative stress in autism

2.3.1 Transmethylation and transsulphuration abnormalities

In two studies, James et al. (230,235) found that, compared to healthy controls, children with autism had significantly lower plasma concentrations of methionine, SAM, homocysteine, cysteine and total glutathione, and significantly higher concentrations of SAH, adenosine and GSSG. All of these changes are characteristic of oxidative stress. Oxidative inactivation of methionine synthase may explain the lower levels of methionine and SAM (230), while the decreased homocysteine levels may reflect compensatory upregulation of the transsulphuration pathway due to a decrease in glutathione synthesis (235). These findings suggest that in autism, oxidative stress is increased, while methylation capacity and redox potential are decreased. In a follow-up study, parents of children with autism showed similarly perturbed oxidative profiles, supporting a possible role for genetics in the development of autism (235). Deficiencies in the transsulphuration pathway are further indicated in several studies. Geier et al. found significantly lower plasma concentrations of cysteine in children with autism, as well as decreased levels of sulphate, taurine, and GSH, all of which are
synthesised from cysteine (236-238). Plasma levels of vitamin B6, an important cofactor in the transsulphuration pathway, are significantly increased in children with autism compared to controls (239). This is likely due to reduced cellular uptake or storage capacity for vitamin B6 within the cell in autism (240). The decreased levels of homocysteine noted previously (230,235) may be worsened by lower cellular levels of vitamin B6, which catalyses the conversion of homocysteine to cysteine via cystathionine.

2.3.2 Consequences of glutathione deficiency

There are a number of systemic abnormalities associated with glutathione deficiency that are commonly found in autism (228). For example, glutathione plays a key role in xenobiotic detoxification (224), and autism is associated with decreased detoxification capacity of phenolic compounds, acetaminophen, and heavy metals (228). Impaired ability to detoxify heavy metals in autism is indicated by studies showing decreased excretion of cadmium, arsenic, and lead (241-243). These metals preferentially react with free sulphhydryl groups, such as those constituting glutathione, and disrupt metabolic processes (244). An excess of heavy metal ions may also inhibit methionine synthesis, thereby limiting glutathione and sulphate production (245), perpetuating the cycle of impaired detoxification.

Glutathione is particularly important for mercury detoxification, as mercury is excreted hepatically after formation of a glutathione-mercury complex in the liver (246). Geier et al. (236) demonstrated a link between decreased mercury detoxification capacity and increased oxidative stress in a study of 28 children with autism, which measured urinary porphyrins and plasma cysteine, reduced glutathione, oxidised glutathione, and total sulphate. Interestingly, several studies have found a positive correlation between the severity of autism symptomatology and the excretion of mercury and other heavy metals (236,247), suggesting that a treatment approach targeting glutathione pathways, thus improving detoxification capacity, may positively impact behavioural symptoms.
Glutathione also plays a role in the maintenance of gastrointestinal and immune functioning (224), both of which have been shown to be impaired in autism (64,248), as noted in Section 2.1.2.

2.3.3 Genetic studies

Several studies have found an increased risk for autism associated with several polymorphisms known to modulate metabolite levels in the transmethylation and transsulphuration pathways (235,249). These include alleles of genes that negatively affect transport of folate and vitamin B12 into cells, as well as the synthesis of metabolically active folate. Vitamin B12 and 5-methyl-tetrahydrofolate, the principal form of folate in the body, are essential for the transmethylation of homocysteine to methionine (240).

A genetic basis for the association between autism and oxidative stress has also been suggested from investigations of polymorphisms of the glutathione-S-transferase gene (250), the metal-regulatory transcription factor 1 gene (251), the glutaredoxin and cystathione gamma lyase genes (252), and the glutathione peroxidase gene (253). In particular, some alleles for these genes may have differential effects on susceptibility to autism, conferring protective effects against autism in some cases (252,253). Genetic links between autism and mitochondrial dysfunction have also been suggested (254-256).

2.3.4 Mitochondrial dysfunction

There is increasing evidence that mitochondrial dysfunction may play a role in autism (257,258). There is a relatively high prevalence of primary mitochondrial disease in children with autism, around 1 in 20 (25), compared to a population prevalence of 1 in 2000 (259,260). Abnormalities in the mitochondrial electron transport chain complexes have been identified in a number of case-control studies and case reports (261), potentially contributing to increased ROS levels and oxidative stress in autism.
Impairments in fatty-acid β-oxidation have been postulated as a contributor in autism pathophysiology, stemming from a case report of a perturbed acyl-carnitine profile in a child with autism (262). Abnormal carnitine, pyruvate, ammonia, and alanine levels may indicate dysfunctions in mitochondrial energy generation. In a chart review of 100 children with autism, Filipek et al. (263) found that 83 cases had total and free carnitine and pyruvate levels below, and ammonia and alanine levels above, the reference mean. However, they did not take into account the maximum and minimum levels for control participants, which may have been a more informative comparison.

Similarly, an elevated lactate/pyruvate ratio may be indicative of a disturbed cellular redox state resulting from mitochondrial dysfunction (259). This is also supported by studies finding elevated lactate in autism (264). Around 20% of children with autism in one study (n=69) showed increased plasma levels of lactate and increased lactate/pyruvate ratio compared to controls (265). Conversely, two large neurochemical studies (266,267) failed to find a difference in lactate levels between children with autism and controls, though this may have been due to questionable sensitivity of the imaging procedures used (268). Both studies did, however, find a significantly reduced concentration of N-acetyl aspartate in children with autism, which is a marker of mitochondrial dysfunction and resulting neuronal damage (269).

2.3.5 Other markers of oxidative stress in autism

Levels of nitric oxide and glutathione peroxidase (GSH-Px) activity are reportedly increased in autism (270-273). Nitric oxide, a free radical, has previously been implicated in the pathophysiology of schizophrenia and bipolar disorder (270). Although its activity is important for a number of biological functions including neurotransmitter release, memory and learning, its reactions with other free radicals yield oxidative by-products, which are associated with lipid peroxidation and cellular damage. Meanwhile, GSH-Px protects cells from oxidative damage by catalysing the reduction of lipid hydroperoxides and hydrogen peroxide. Activity of this enzyme may increase under conditions of oxidative stress as compensation for increased lipid peroxidation (270,271). As GSH is consumed in GSH-Px-catalysed reactions, this may further deplete GSH levels (271).
Studies of red blood cell enzymes in autism have shown increased activity of xanthine oxidase (XO) and the free radical-scavenging enzyme superoxide dismutase (SOD), and decreased catalase activity (271-274). Xanthine oxidase is essential for the conversion of xanthine into uric acid, which acts as a pro-oxidant at high levels and generates free radical superoxide as a by-product. Superoxide dismutase catalyses the conversion of superoxide into hydrogen peroxide, while catalase converts hydrogen peroxide into oxygen and water.

Based on findings of increased SOD activity and decreased catalase activity, levels of hydrogen peroxide, a highly toxic ROS, are significantly elevated in people with autism, indicating the presence of oxidative stress (271,274). The authors suggest that, like GSH-Px, SOD activity may be increased as a compensatory mechanism against oxidative stress (274), but the decrease in catalase activity may mean that the hydrogen peroxide produced from SOD activity is not adequately metabolised (271).

Contrary findings were reported by Yorbik et al. (275), who found decreased activity of SOD and GSH-Px in plasma and red blood cells of children with autism, and suggest that this may be a cause, rather than a consequence, of pathological changes in autism. They posit that decreased SOD activity may result in increased superoxide levels, which may in turn lead to DNA damage, impacting neuronal growth and migration. Likewise, accumulation of free radicals resulting from decreased GSH-Px activity may lead to alterations in gene expression (275). The inconsistency between the data from this study and those from the previously mentioned studies (271,274) may be due to methodological or sample differences; nonetheless, changes in antioxidant enzyme activity seem to be related to the pathophysiology of autism, and may correlate with severity scores on behavioural measures (273).

2.3.6 Summary

A number of oxidative biomarkers are altered in people with autism, and genetic links have been found between abnormalities in redox status and autism. While there is substantial evidence demonstrating that oxidative pathways are disturbed in autism, it is undetermined whether oxidative stress is a cause of autism, contributes to the illness, or
is a consequence of the illness. If oxidative stress has a causal relationship with autism, it is also undetermined whether this relationship is ongoing or only occurs during the onset of the illness.

Ultimately, it is likely that autism arises from a combination of genetic and environmental factors (190,208,210). Early exposure to certain environmental toxins may simultaneously increase the need for glutathione, a major xenobiotic detoxifier, while restricting glutathione synthesis (228). In people with pre-existing genetic vulnerability (e.g. decreased detoxification abilities), this may lead to accumulation of toxins, eventually resulting in oxidative stress (228). Neurons are particularly susceptible to oxidative damage (223), with the resulting neuronal cell death and decreased synaptic efficiency possibly playing a role in the pathophysiology of autism. Impaired energy generation and immune dysfunction associated with oxidative stress may also contribute to the clinical presentation of autism (218).

2.4 Antioxidant therapy in autism

2.4.1 Research to date

Several treatments targeting redox pathways have been investigated based on the accumulating evidence of oxidative stress involved in the pathophysiology of autism (4). In an open-label trial of methylcobalamin and folinic acid in children with autism (n=48, mean age=5.1 years) (276), improvements on all subscales of the Vineland Adaptive Behavior Scales (Vineland-II) – Communication, Daily Living and Socialisation – were shown. The improvements shown in this study translated to an average of a 7-month gain in age-equivalent skills across the 3-month treatment period. Outcomes were moderated by change in GSH/GSSG ratio, suggesting that improvements were due to an improvement in redox status. This treatment regime may have promoted cysteine and glutathione synthesis by acting on both transmethylation pathways: firstly, treatment with folinic acid provided folate for the methionine synthase-catalysed transmethylation pathway; secondly, betaine acted as the precursor for the BHMT-catalysed pathway (refer to Figure 2.1). However, as with all open-label
studies, this result may have been significantly influenced by expectation bias, placebo effects and regression to the mean.

One third of children in a small RCT of methylcobalamin ($n=30$, age range=3-8 years) (277) showed significant improvements in cognitive and behavioural outcomes, following twice-weekly administration of methylcobalamin ($64.5 \mu g/kg$). This group improved on measures of communication and problem behaviour, and showed increased levels of GSH and GSH/GSSG ratio. However, across the entire sample, differences between treatment and placebo were non-significant. This study used a cross-over design, where participants randomly received either methylcobalamin or placebo for six weeks and were then crossed over for a further six weeks, with no washout period between the two phases. It is possible that some participants who received methylcobalamin first may have experienced improvements which continued into the subsequent placebo phase, contributing to the lack of overall treatment effect (277).

Carnosine, a known scavenger of ROS and lipid peroxidation by-products (278), has been proposed as a treatment for Alzheimer’s disease, with preliminary findings suggesting benefits due to its antioxidant and homeostatic properties (279). Treatment of autism with carnosine, at a dose of 400 mg twice daily for eight weeks, improved performance on measures of language and socialisation in a small RCT ($n=31$, age range=3-12 years) (280).

### 2.4.2 Glutathione as a therapeutic target: N-acetylcysteine

Studies reviewed in Section 2.3 have demonstrated a link between glutathione deficiency and pathology associated with autism, though it is currently unknown whether this link is causative. Oxidative stress and glutathione deficiency have been found in a number of psychiatric illnesses (215), suggesting that these may be important contributors to psychopathology in a range of disorders, and that treatments targeting glutathione synthesis may be effective in improving these conditions (223).

Cysteine is the rate-limiting agent in glutathione synthesis (229). Levels of free cysteine in the body are relatively low, as it is readily oxidised to form cystine (224); direct oral
supplementation of cysteine is mostly ineffective for this reason (281). Administration of oral N-acetyl cysteine (NAC) is considered a more effective method of replenishing cysteine levels (281). The presence of the acetyl group protects cysteine from oxidation until it is absorbed into the liver and deacetylation occurs, ensuring the availability of cysteine for intracellular glutathione synthesis (223). Its half-life is estimated at two to three hours in adults when orally administered, with oral bioavailability of around 6-10% (282).

N-acetyl cysteine is currently indicated for treatment of acetaminophen (paracetamol) overdose and as a mucolytic (283), though it has been trialled in a range of clinical pathologies (227). Intravenous administration of NAC is associated with some adverse effects at high doses, including anaphylaxis and hypotension (281), while oral administration of NAC is considered safe and tolerable at doses up to 8000 mg daily in adults. Side effects of oral NAC may include mild nausea, vomiting and diarrhoea (283). Its properties in children are less established, particularly the safety of long-term administration.

2.4.2.1 NAC in psychiatry

The efficacy of NAC has been investigated in a range of psychiatric conditions and disorders, including schizophrenia; mood disorders, such as major depressive disorder and bipolar disorder; addiction; and impulse control disorders, such as trichotillomania (227). Reportedly, many of these disorders are associated with similar patterns of oxidative stress to autism (223,227).

In a double-blind RCT, Berk et al. (5) demonstrated the efficacy of NAC, at a dose of 1000 mg twice daily for 24 weeks, in improving symptoms in schizophrenia (n=140, mean age=36.6 years). Specifically, improvements were shown in negative symptoms (e.g. anhedonia and asociality), as measured by the Positive and Negative Syndrome Scale (PANSS) and in overall clinical global impression (5). The findings of improved scores on the PANSS have since been replicated in a smaller RCT (284), and there are suggestions from RCTs that NAC may also enhance auditory processing and EEG synchronisation in people with schizophrenia (285,286).
In an RCT of NAC in bipolar disorder (n=75, mean age=45.6 years), participants showed improvements on measures of depressive and manic symptoms, overall functioning and quality of life (6). In this and the schizophrenia trial by Berk et al. (5,6), differences between NAC and placebo groups largely disappeared four weeks after treatment discontinuation. The authors suggest that given the rapid turnover of glutathione, particularly under conditions of oxidative stress, continual supplementation with NAC may be necessary to maintain clinical benefits (5).

NAC has been shown to be effective in improving depressive symptoms, across a range of psychiatric diagnoses, at a meta-analytic level of evidence (287). To date, only one RCT has been conducted in major depressive disorder (n=252, mean age=50.2 years), which found that NAC was associated with significant improvements in global functioning during the trial period, and in depressive symptoms at post-discontinuation follow-up (288), in contrast with the schizophrenia and bipolar disorder trials where effects of NAC did not continue post-discontinuation.

The efficacy of NAC in addictive disorders has also been investigated in a number of RCTs and crossover placebo-controlled trials, including cannabis, cocaine, methamphetamine, nicotine and gambling addiction, reviewed by Deepmala et al. (227). There is also growing interest in the use of NAC in impulse control disorders, such as trichotillomania. In their systematic review of NAC in these disorders, Deepmala et al. (227) report that the evidence to date is mixed but warrants further investigation.

2.4.2.2 NAC in autism

Initial case studies of NAC in children with autism reported encouraging findings (289,290). The first case, an 8-year-old boy receiving 800 mg daily of NAC for two months, showed improvements in verbal communication and social interaction, and reductions in nail biting, aggression, and stereotypies, according to parent report (289). The second, a 4-year-old boy receiving 1800 mg daily of NAC, ceased self-injurious behaviour within two months, with the behaviour returning during a short period without NAC and subsiding again after NAC was reintroduced (290).
Four pilot RCTs, conducted contemporaneously with the current study, investigated the use of NAC in children with autism. The first, reported in 2012 by Hardan et al. (7), investigated NAC in addition to treatment as usual in children with autism (n=33, age range=3-10 years). This study found that compared to placebo, NAC significantly improved symptoms of irritability, social cognition, and autism mannerisms. Outcomes were measured using the Aberrant Behavior Checklist (ABC). Participants received an increasing dose of NAC across 12 weeks, beginning at 900 mg once daily and increasing every four weeks to an eventual dose of 900 mg three times daily. For the primary outcome, irritability, the largest difference between the NAC and placebo groups was seen at week 4, though improvement did continue at a slower pace in the NAC group over the remaining eight weeks. There was no difference between NAC and placebo in safety and tolerability.

The second pilot study, reported in 2013 by Ghanizadeh et al. (8), was an 8-week placebo-controlled, randomised trial of NAC in addition to risperidone (n=40, age range=3-16 years). All participants received 2-3 mg daily of risperidone depending on body weight, with 600 mg of NAC or matched placebo twice daily (1200 mg total daily dose). This study found that NAC treatment did not alter the primary outcome, total scores on the ABC, but did improve scores on the irritability subscale. There were no changes in stereotypic behaviour, communication, or social interaction. There were a greater number of adverse events reported in the NAC group, including drowsiness, constipation, and nervousness; however, significance testing was not reported for frequency and severity of adverse events. All adverse events were considered mild.

These findings were replicated by Nikoo et al. (9), in their 10-week, randomised controlled trial of NAC in addition to risperidone (n=40, age range=4-12 years). Dosage was 200-300 mg of NAC or matched placebo twice daily, in addition to 1-2 mg daily of risperidone, depending on body weight. As with the previous two trials, participants in the NAC group showed a significantly greater improvement on the irritability subscale of the ABC than the placebo group. Additionally, NAC was associated with higher improvement on the hyperactivity/non-compliance subscale of the ABC. There was no significant difference in the incidence of adverse events between NAC and placebo.
Finally, a recently published study by Wink et al. (10) was the first to investigate the physiological effects of NAC in children with autism, in a 12-week placebo-controlled randomised trial (n=31, age range=4-12 years). Dosage was 60 mg/kg/day of NAC or matched placebo, administered in three equal doses daily. As expected, the study found that GSH and GSSG levels increased in the NAC group compared to the placebo group, though the difference only reached significance for GSH levels. There were no significant differences on GSH/GSSG ratio, homocysteine levels or measures of oxidative damage to DNA. In addition, there were no differences found on any behavioural measures, including the ABC (10).

These results indicate that NAC supplementation may be safe, tolerable and effective in treating irritability in children with autism, but not the core symptoms of autism – communication, social interaction, and repetitive or stereotyped behaviours or interests. However, the small sample size and relatively short duration of these studies indicated that further investigation was warranted (7).

2.5 The current study

2.5.1 Aims and hypotheses

The aim of the current study was to investigate the safety and efficacy of oral NAC in treating children with autism in a double-blind, randomised, placebo-controlled trial. Outcome measures included mixed-methods assessments of the core symptoms of autism – communication, social interaction, and repetitive or stereotyped behaviours or interests – as well as measures of problem behaviours and daily functioning.

It was hypothesised that 500 mg daily of NAC treatment, in addition to treatment as usual, would be associated with a greater improvement, compared to placebo, in core autism symptomatology as measured by the Social Responsiveness Scale (SRS) (291), Children’s Communication Checklist – Second Edition (CCC-2) (292), and the Repetitive Behavior Scale – Revised (RBS-R) (293). It was also hypothesised that NAC would be similar to placebo in terms of safety and tolerability.
It was also hypothesised that, compared to placebo, NAC would be associated with a greater improvement in: behavioural problems, as measured by the Developmental Behaviour Checklist – Primary Carer Version (DBC-P)(294); adaptive functioning, as measured by the Vineland Adaptive Behavior Scales – Second Edition (Vineland-II)(295); and three global impression scales (296), known as the Parent Global Impression – Improvement scale (PGI-I), Clinical Global Impression – Improvement scale (CGI-I), and Clinical Global Impression – Severity scale (CGI-S).

This study also aimed to supplement the main efficacy study with an exploratory qualitative analysis of parent/guardian reports and clinicians’ observations over the course of the study.

2.5.2 Summary

Based on evidence that oxidative stress, particularly glutathione deficiency and dysfunction, may play a role in the pathophysiology of autism, this study aimed to investigate the efficacy of NAC in improving behavioural symptoms in children with autism. The next chapter will detail the methods and measures used in this study.
Chapter 3. Methods

3.1 Study design and administration

This study was a multi-site, mixed-methods, placebo-controlled, randomised clinical trial of 500 mg daily N-acetyl cysteine (NAC), in addition to treatment as usual, in children with a *DSM-IV-TR* (2) diagnosis of autistic disorder (henceforth referred to as ‘autism’). The trial was conducted according to Good Clinical Research Practice guidelines (297) and funded by an Australian Rotary Health Mental Health Research Pilot Project grant (2010-2011) and a Simons Foundation Autism Research Initiative grant (2012-2014).

Approval was received from the Human Research Ethics Committees (HRECs) at Barwon Health (primary site), Monash University, University of Melbourne and Deakin University. The primary HREC approval letter is included in Appendix A. The trial was registered with the Australian & New Zealand Clinical Trials Registry (registration number: ACTRN12610000635066) and the Therapeutic Goods Administration of Australia.

3.2 Site feasibility

The study was conducted at two sites in Victoria, Australia: the Centre for Developmental Psychiatry and Psychology (CDPP), Monash University, Clayton; and Barwon Health including Gateways Support Services, Geelong.

3.2.1 The Barwon region

The Barwon region of south-western Victoria includes Geelong, the state’s second largest city, and the surrounding rural area, encompassing a relatively stable population of approximately 280,000 people (298). A 2004 study concluded that the prevalence of ASDs in the Barwon region was approximately 39 in 10,000, with around half of these being diagnoses of autism (23).
The Barwon study site recruited from the Gateways Support Services, based in Geelong West. Gateways is the major provider of specialist services for individuals with autism in the Barwon region. Through Gateways, families access diagnostic services, early intervention, integration support for childcare and kindergarten, parent support groups, respite care, and social recreation programs for children, teenagers and adults.

3.2.2 Greater Melbourne

Melbourne is Australia’s second largest city, and the capital and largest city of Victoria, with a diverse population of approximately 4 million. The prevalence of ASDs in Victoria, across metropolitan and rural regions, has been estimated at 27 in 10,000 among 0-6 year olds (299), though there has not yet been a systematic study of the prevalence in Melbourne alone.

There is a wide range of publicly funded and private facilities available for individuals with autism in greater Melbourne, including diagnostic services, providers of various interventions, and community support and information groups. The Melbourne study site was the Centre for Developmental Psychiatry and Psychology (CDPP), Monash University, Clayton, in the southeast suburbs of Melbourne. The CDPP conducts research and provides clinical and educational services, focusing on neurodevelopmental and genetic conditions in children and adolescents, including autism, attention-deficit hyperactivity disorder, and intellectual disability.

3.3 Investigational product

The investigational product in this study was 500 mg N-acetyl cysteine (NAC) or matching placebo capsules. N-acetyl cysteine (empirical formula C₅H₉NO₃S) is a white, crystalline powder. Placebo capsules contained corn starch. NAC and placebo capsules were identical in size and appearance, and were stored in identical bottles. The outside of the placebo capsules was lightly dusted with a small amount of NAC to approximate the smell of the active capsules and assist in keeping participants and their families blinded.
The fixed dose of 500 mg once daily was selected due to the absence of safety data on the long-term administration of NAC in children, at the time that this study was conceived. Previous trials in adults had used doses of around 2000 mg daily (5,6). Given the younger age and lower body weight of the target population in the current study, this dose was deemed an acceptably safe choice by the investigators and Barwon Health HREC.

### 3.3.1 Acquisition

The investigational product was supplied in two batches for this trial due to changes in funding. The first batch was manufactured by the Zambon Group in Italy and encapsulated by DFC Thompson in Sydney, New South Wales, Australia; trial packs were made in the Barwon Health pharmacy in Geelong, Victoria, Australia. The second batch was manufactured by BioMedica in Sydney, New South Wales, Australia; and capsulated and packed at Dartnell’s Pharmacy in Surrey Hills, Victoria, Australia. Certificates of analysis, provided by the manufacturers, confirmed that the investigational product met all required specifications for stability and purity, and was comparable across batches.

### 3.3.2 Product storage and stability

Before dispensing to participants, the investigational product was stored in a restricted area that only project staff members were able to access (i.e. Barwon Health pharmacy or locked office). The temperature of the storage space was maintained between 15 and 25 degrees Celsius, with temperature monitored and logged daily. Upon dispensing, parents/guardians were instructed to store the capsules in a cool, dry place out of reach of children.

### 3.3.3 Accountability procedures

The investigational product was dispensed from the Barwon Health pharmacy. A master capsule log, kept at the Barwon Health pharmacy, was used to track the number of capsule packs stored and dispensed. The trial packs were dispensed to participants at
baseline, 1 and 3 months and this was logged on an individual capsule log, listing the randomisation number, date, and number of capsules dispensed.

At 1, 3 and 6 months, participants’ parents/guardians were asked to return their previous capsule bottles, along with any unused capsules. The date of return and the number of remaining capsules are logged on the same individual capsule log. Taking into account the number of days between the date of dispensing and the date of return, this was intended to give a measure of protocol adherence. Audited, returned capsule bottles and any unused capsules were destroyed at the end of the trial.

3.4 Participants

3.4.1 Recruitment and screening

Participants were recruited through a multi-pronged strategy, including advertisements through autism diagnostic services, including the CDPP and Gateways Support Services; autism support services including Autism Victoria (now trading as Amaze); general community health services; and the wider media. Specific recruitment avenues included news articles, press releases and paid advertisements in local and state-wide newspapers, advertisements in newsletters and on websites of family support networks, mail-outs from autism diagnostic and support services, and flyers placed in waiting rooms of paediatricians and autism diagnostic and support services.

Interested parents/guardians of prospective participants were invited to contact the researchers directly. Initial contact was made by telephone interview or email, with information provided to participants including the relevant inclusion criteria (correct age range and diagnosis of autism). The Participant Information and Consent Form (PICF) was then sent to parents/guardians to read. Parents/guardians were encouraged to discuss the study with their family and paediatrician before agreeing to participate.

Parents/guardians interested in proceeding were telephoned to follow up their potential participation. During this telephone call, the exclusion criteria were reiterated to prevent parents/guardians and children attending an interview for which they were ineligible.
Parents/guardians were asked to submit their child’s diagnostic and/or assessment reports for review. Two experienced child psychologists reviewed these reports in order to confirm that all participants met diagnostic criteria for autism before admission to the study.

Participants and their parents/guardians were asked to attend a baseline appointment with a trial clinician at one of the study sites. The trial clinician confirmed that the parent/guardian had read and understood the PICF, and answered any questions they had regarding participation, before obtaining formal written consent at the beginning of the baseline appointment. The PICF is included in full in Appendix B.

3.4.2 Inclusion criteria

Children were eligible for participation in this study if they:

(i) Had a diagnosis of autistic disorder according to DSM-IV-TR criteria (2), as confirmed by case review;

(ii) Were aged between 3 and 10 years inclusive upon entry to the study; and

(iii) Had reached a stable dose (stable for at least two weeks prior to study commencement) of any pharmacotherapies they were receiving.

3.4.3 Exclusion criteria

Individuals were excluded from the study if, based on both parent/guardian report and clinical review, they:

(i) Had a primary diagnosis of any other disorder that was not autistic disorder, according to DSM-IV-TR criteria (2);

(ii) Had a known or suspected clinically relevant systemic medical disorder or known genetic or metabolic cause of developmental delay;

(iii) Had a prior sensitivity or allergy to NAC;

(iv) Were considered likely to be unable to comply with the treatment protocol, e.g. having a highly restricted diet leading to refusal to take NAC or placebo capsules;
(v) Had parents/guardians who were non-fluent in English, due to the majority of outcome measures relying on parent report;
(vi) Had a history of asthma, as asthma is a known risk factor for adverse events relating to NAC (300);
(vii) Had a diagnosis of epilepsy or history of seizures, as NAC has been shown to reduce seizure threshold in susceptible persons (301); or
(viii) Were already receiving any treatment containing NAC, glutathione or their precursors.

3.5 Study procedures

3.5.1 Participant flow

At the baseline appointment, participants were randomised sequentially into either the NAC or placebo group. The randomisation code was generated by a computer program designed for clinical trial randomisation (www.randomization.com). Treatment and placebo packs were randomly allocated in blocks of four, with a treatment/placebo ratio of 1:1. An independent researcher, who had no contact with any participants, coordinated the randomisation codes. Participants, their parents/guardians, statistical support staff, and study clinicians were blind to block size and treatment arm allocation for the duration of the study.

Figure 3.1 displays the study visit schedule. Participants and their parents/guardians attended appointments at baseline, 1, 3 and 6 months during the treatment phase, and post-treatment follow-up visits at 12 and 18 months. Due to the timeframe in which the post-treatment visits were completed, only data from the 6-month treatment phase is reported in this thesis.

Participants were seen in clinical rooms designed for the assessment and interview of children and their families. At each visit during the treatment phase, parent/guardians received a bottle of NAC or placebo capsules: 35 capsules at baseline, 70 at 1 month, and 105 at 3 months. For each participant, the first dose of NAC or placebo was
Figure 3.1. Timeline of study visits
administered at the baseline visit, in order to monitor for any acute reactions in a supervised environment.

Parents/guardians were given detailed directions regarding storage and method and frequency of delivery of the capsules. Throughout the treatment phase, doses were to be administered orally once daily to participants, at approximately the same time each day. If a participant was unable or unwilling to swallow the capsules whole, parents/guardians were advised to empty and stir the capsule contents into a beverage, such as orange juice or chocolate flavoured milk. Any unused capsules were returned at the next visit to assess adherence to the trial protocol.

Phone interviews were conducted at 2, 4 and 5 months to monitor for any adverse events between appointments, in addition to the detailed questioning about adverse events and medical issues that took place at face to face visits. Any concerns regarding safety, tolerability and withdrawal of participants were discussed at fortnightly team meetings. In the event of urgent safety concerns, procedures were in place to refer participants to immediate medical treatment, with unblinding occurring if deemed medically necessary.

3.5.2 Withdrawal criteria

Participants were withdrawn from the study if any of the following criteria applied:

(i) They ceased taking the investigational product for seven consecutive days or more;
(ii) Parents/guardians withdrew consent; or
(iii) A serious adverse event occurred which impacted their participation.

Withdrawal due to adverse events could be either at the request of the parent/guardian or the discretion of the investigator.

There were no restrictions on commencing, changing or ceasing pharmacological or behavioural therapies throughout the course of the study.
3.5.3 Data management

Raw data was collected in participants’ case report forms (CRFs), which were kept in a locked filing cabinet in an office at Barwon Health or the CDPP, which only trial personnel were able to access. De-identified data were entered electronically into a locked Microsoft Excel file, again with restricted access. Records relating to the results of the study are to be kept for 23 years in secure archives at Barwon Health.

3.6 Study measures

The primary outcome measures were the Social Responsiveness Scale (SRS) (291), Children’s Communication Checklist – Second Edition (CCC-2) (292) and the Repetitive Behavior Scale – Revised (RBS-R) (293), along with their associated subscales. During the treatment phase, an adverse events checklist was completed on a monthly basis post-baseline to monitor adverse events and safety concerns. Questionnaires were completed by parents/guardians at baseline, 1, 3 and 6 months. Unless there were prohibiting circumstances, the same parent/guardian completed all questionnaires and attended all interviews.

At baseline only, a demographic interview, designed specifically for this study, was completed, along with the parent-completed Social Communication Questionnaire (SCQ) – Lifetime and Current versions (302). A subset of participants also completed the clinician-administered Autism Diagnostic Observation Schedule (ADOS) (303) and an age-appropriate cognitive assessment, the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III) (304) for children aged up to seven years, or the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV) (305) for children aged over seven years. Together, the SCQ, ADOS and cognitive assessment provided a comprehensive picture of participants’ social and intellectual functioning at baseline.

Secondary outcomes were the following measures of behaviour problems, adaptive behaviour and overall functioning: Developmental Behaviour Checklist – Primary Carer Version (DBC-P) (294); Vineland Adaptive Behavior Scales – Second Edition
(Vineland-II) (295); and three global impression scales (296) known as the Parent Global Impression – Improvement scale (PGI-I), Clinical Global Impression – Improvement scale (CGI-I), and Clinical Global Impression – Severity scale (CGI-S). The subscales of the DBC-P and domain scores on the Vineland-II were also secondary outcomes. The DBC-P was completed by parents/guardians at all visits. The Vineland-II was completed at 6-monthly intervals only. As the PGI-I and CGI-I required parents/guardians and clinicians, respectively, to rate changes in the child since baseline, this scale was completed at all visits except baseline. Clinicians completed the CGI-S at all visits.

At all appointments, study clinicians took summary notes and verbatim quotes where possible, based on parent/guardian interview and direct observation of the participant, for later qualitative analysis. Qualitative analysis was conducted by an honours candidate who was blind to treatment arm (see Section 3.8).

All interview forms and outcome measures are included in full in Appendix C, with the exception of the Vineland-II due to its length. Table 3.1 outlines the measures administered at each visit during the 6-month trial period. A detailed description of all scales completed as part of this study follows.

### 3.6.1 Baseline assessments

#### 3.6.1.1 Demographic interview

Demographic data was collected at baseline by the clinician in a semi-structured interview, developed specifically for this study. The interview included questions on: developmental and medical history; past and present autism interventions and medications (including complementary and alternative treatments); education; history of regression (i.e. loss of pre-existing language or other skills); family history of autism and other developmental disorders; and parent/guardian socio-economic data. A complete copy of this interview is included in Appendix C.
### Table 3.1. Measures completed at each visit

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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Social Communication Questionnaire – Lifetime and Current</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cognitive assessment (WPPSI-III/ WISC-IV)</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Autism Diagnostic Observation Schedule</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Secondary and other measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Update interview</td>
<td>-</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Developmental Behaviour Checklist – Primary Carer Version</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Vineland Adaptive Behavior Scales</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>P</td>
</tr>
<tr>
<td>Clinical Global Impression – Severity</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Clinical Global Impression – Improvement</td>
<td>-</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Parent Global Impression - Improvement</td>
<td>-</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

3.6.1.2 Social Communication Questionnaire (SCQ) – Lifetime and Current

The SCQ was developed by Rutter and Lord (302) as a measure of autism symptomatology and a screening tool for ASD. The Lifetime version taps the developmental history of the individual, while the Current version refers to the individual’s behaviour in the past three months. Each questionnaire consists of 40 yes/no questions, including items specifically relating to reciprocal social interaction (e.g. ‘did/does she/he ever show you things that interested him/her to engage your attention?’); communication (e.g. ‘can you have a to and fro “conversation” with her/him that involves taking turns or building on what you have said?’); and restricted, repetitive and/or stereotyped patterns of behaviour (e.g. ‘has she/he ever had any mannerisms or odd ways of moving her/his hands or fingers, such as flapping or moving her/his fingers in front of her/his eyes?’).

Responses indicating the presence of an abnormal behaviour are scored 1, while responses indicating their absence are scored 0. A total score of 15 or above, out of a possible 39, on the Lifetime questionnaire is considered a positive screen for an ASD (302). This cut-off has been established to have reasonable sensitivity and specificity in distinguishing between children with and without autism (306).

3.6.1.3 Autism Diagnostic Observation Schedule (ADOS)

The study was designed to confirm diagnosis based on case review and it was expected that all children who met criteria for the study would have previous ADOS and cognitive assessment results available. However, it became apparent during the course of the study that this was not always the case, either because the assessments were not completed or these reports could not be provided by the parent/guardian. As such, while participant diagnosis was confirmed through case review only for the first 21 participants, the ADOS was completed at baseline with all subsequently enrolled participants (n=77), as well as cognitive testing (detailed in the next section). The ADOS was used to provide additional confirmation of diagnosis and a clinician-rated measure of symptom severity.
The ADOS is a standardised assessment which uses a series of structured play- or conversation-based activities to assess social interaction, communication, play and stereotyped or repetitive behaviour or interests (307). Developed from empirical research, the ADOS provides a variety of standardised scenarios, appropriate to an individual’s developmental and verbal levels, as a prompt for social initiations and responses. One of four modules may be administered: module 1 for children without consistent phrase speech, module 2 for children with some flexible phrase speech, module 3 for verbally fluent children, and module 4 for adolescents and adults. Only modules 1-3 were used in this study.

ADOS scores, ranging from 0 to 3 for some items and 0 to 2 for others, are given based on the frequency or severity of behaviours observed during the assessment, such as unusual eye contact, facial expressions directed to others, stereotyped/idiosyncratic use of words or phrases, and spontaneous initiation of joint attention. A score of 0 for an item indicates the absence of abnormal behaviour, while a positive score indicates its presence and severity.

Summary scores in impairments in communication and social interaction (Social Affect), and stereotyped behaviours or restricted interests (Restricted and Repetitive Behaviours), are produced by the sum of salient items in each domain. An overall severity score is derived from their total sum (308). The severity score has high sensitivity (up to r=.98) and specificity (up to r=.94) in distinguishing between children with a diagnosis of autism or other ASD and those without (309). The ADOS is established to have high inter-rater reliability across individual items (over 80% agreement for all items) and for diagnostic classification (up to 100%), with good to excellent test-retest reliability (303). Together with parent interviews and other assessments, the ADOS is an important tool in the diagnosis of autism and other ASDs. It was used in this study to confirm participants’ diagnoses of autism, and to provide a baseline, clinician-rated measure of the severity of autism symptomatology.
3.6.1.4 Cognitive assessments

During the latter part of the study (as described in the previous section), cognitive assessments were conducted at baseline for participants without a current assessment (i.e. had not completed a cognitive assessment in the last two years). In total, cognitive assessments were completed with 49 participants. Current assessment results were available for an additional 33 participants from other sources. Depending on the age of each participant, either the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III) or the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV) was completed.

The WPPSI-III has been developed for use in children aged between 2 years, 6 months and 7 years, 3 months (304). It comprises 14 subtests in the categories of verbal and performance (non-verbal) abilities. Verbal subtests include Receptive Vocabulary (where the child points to a named picture out of a group of four) and Comprehension (where the child orally answers questions about common objects or situations). Performance subtests include Block Design (where the child copies specific designs using multi-patterned blocks) and Object Assembly (where the child completes puzzles of increasing difficulty). Children aged less than four years complete only a subset of these tasks.

Summary scores generated by the WPPSI-III include age-standardised subtest scores, which have a mean of 10 and a standard deviation of 3. Performance IQ (PIQ) and Verbal IQ (VIQ) can then be derived based on performance and verbal subtest scores respectively, with age-standardised means of 100 and standard deviations of 15 (304). The Full Scale IQ (FSIQ) provides an overall standardised measure of intellectual functioning. Split-half reliability of the WPPSI-III subtests is between .79 and .92 in an Australian sample. Construct validity is indicated by exploratory factor analysis showing strong factor loadings (.41-.88) of the Verbal and Performance subtests onto their corresponding factors (304).

The WISC-IV is designed for children aged between 6 and 16 years (305). Eleven of the WISC-IV’s 15 subtests are equivalent to those in the WPPSI-III, with four age-
appropriate subtests added. In addition to age-standardised subtest scores, with a mean of 10 and a standard deviation of 3, overall summary scores include composite measures of Verbal Comprehension (VCI), Perceptual Reasoning (PRI), Working Memory, and Processing Speed, with age-standardised means of 100 and standard deviations of 15 (305). As with the WPPSI-III, the Full Scale IQ (FSIQ) provides an overall standardised measure of intellectual functioning.

Like the WPPSI-III, exploratory factor analysis has demonstrated the construct validity of the WISC-IV, with strong factor loadings of each subtest onto their designated composite measures. Internal consistency is shown by high internal reliability for the WISC-IV subtests ($r=.75-.89$) and the four composite measures ($r=.85-.95$). These measures also have good test-retest reliability (305). These results indicate that WPPSI-III and WISC-IV summary scores provide a good overview of each participant’s cognitive functioning in verbal and non-verbal areas.

3.6.2 Primary outcome measures

3.6.2.1 Social Responsiveness Scale (SRS)

The SRS was developed as a tool to assess autism symptomatology and assist in differentiating between diagnoses on the autism spectrum (291). It consists of 65 items relating to five subscales: social awareness, social cognition, social communication, social motivation, and autistic mannerisms, as described in Table 3.2. While the subscales have strong internal consistency (310,311), several studies suggest that the SRS more likely conforms to a one- or two-factor model (312,313). SRS subscale scores are nonetheless reported for this study, due to their conceptual basis and clinical utility.

Items are rated on a 4-point Likert scale from 1='not true' to 4='almost always true', with 17 positive items (e.g. ‘is aware of what others are thinking or feeling’) reverse scored so that scores increase with the presence of abnormal behaviours or the absence of normal behaviours (291). Total scores on the SRS above 87, out of a possible 195, are considered to be in the severe range of autistic symptomatology, while scores between 54 and 86 are considered to be in the mild to moderate range. Scores of 53 or
Table 3.2. Subscales of the Social Responsiveness Scale and example items

<table>
<thead>
<tr>
<th>SRS Subscales</th>
<th>No. of items</th>
<th>Example Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social awareness</td>
<td>8</td>
<td>Walks in between two people who are talking</td>
</tr>
<tr>
<td>Social cognition</td>
<td>12</td>
<td>Is able to understand the meaning of other people’s tone of voice and facial expressions (reverse scored)</td>
</tr>
<tr>
<td>Social communication</td>
<td>22</td>
<td>Has trouble keeping up with the flow of a normal conversation</td>
</tr>
<tr>
<td>Social motivation</td>
<td>11</td>
<td>Would rather be alone than with others</td>
</tr>
<tr>
<td>Autistic mannerisms</td>
<td>12</td>
<td>Has repetitive, odd behaviours such as hand flapping or rocking</td>
</tr>
</tbody>
</table>

Source: *The Social Responsiveness Scale*. Western Psychological Services; 2002 (291).
below are likely to indicate the absence of an ASD (291). The SRS has high internal consistency \((r=.91-.97)\), high inter-rater reliability \((r=.76-.95)\) and stability of repeated measures \((r=.77-.97)\) (291,314). SRS scores also correlate strongly with scores on established autism screening measures, such as the Autism Diagnostic Interview – Revised (315). In this study, the SRS was completed by parents/guardians at every visit to give a measure of severity of autism symptomatology over the course of the trial.

3.6.2.2 Children’s Communication Checklist – Second Edition (CCC-2)

The CCC-2 was developed as a measure of language impairment and communication problems, including those commonly associated with ASDs (292,316). It was completed by parents/guardians at every visit, to capture changes in language and communication over the course of the trial. The CCC-2 consists of 70 Likert items, rated on a scale from 0=’less than once a week (or never)’ to 3=’several times (more than twice) a day (or always)’. Items relate to ten subscales: speech, syntax, semantics, coherence, inappropriate initiation, stereotyped language, use of context, nonverbal communication, social relations, and interests (292). Table 3.3 lists example items for these subscales.

Completion of the CCC-2 yields standardised scores for each subscale with a mean of 10 and standard deviation of 3, providing information about a child’s strengths and weaknesses in communication relative to other children their age. The General Communication Composite (GCC), the sum of the first eight subscale scores, is an overall indicator of severe communication deficits, while the Social Interaction Deviance Composite (SIDC) is calculated as the difference between the subscales relating to social and pragmatic aspects of language, and gives an indication of the pattern of communication deficits (292).

The CCC-2 subscales have high internal consistency \((r=.66-.80)\) and the SIDD in particular has good inter-rater reliability \((r=.79)\). Meanwhile, test-retest reliability is between \(r=.86\) and \(r=.93\) across 1 to 28 days (292). The GCC and SIDC are demonstrably sensitive measures in identifying communication difficulties and pragmatic language impairments in children with ASDs (292).
<table>
<thead>
<tr>
<th>CCC-2 Subscale</th>
<th>No. of items</th>
<th>Example Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speech</td>
<td>7</td>
<td>Speaks fluently and clearly, pronouncing all speech sounds accurately and without any hesitation (reverse scored)</td>
</tr>
<tr>
<td>Syntax</td>
<td>7</td>
<td>Gets mixed up between he and she so might say “he” when talking about a girl</td>
</tr>
<tr>
<td>Semantics</td>
<td>7</td>
<td>Mixes up words of similar meaning, e.g. might say “dog” for “fox”</td>
</tr>
<tr>
<td>Coherence</td>
<td>7</td>
<td>Can be hard to tell if s/he is talking about something real or make-believe</td>
</tr>
<tr>
<td>Inappropriate initiation</td>
<td>7</td>
<td>Asks a question, even though s/he has been given the answer</td>
</tr>
<tr>
<td>Stereotyped language</td>
<td>7</td>
<td>Repeats back what others have just said, e.g. if asked “what did you eat?” might say, “what did I eat?”</td>
</tr>
<tr>
<td>Use of context</td>
<td>7</td>
<td>Misses the point of jokes and puns (though may be amused by nonverbal humour such as slapstick)</td>
</tr>
<tr>
<td>Nonverbal communication</td>
<td>7</td>
<td>Makes good use of gestures to get his/her meaning across (reverse scored)</td>
</tr>
<tr>
<td>Social relations</td>
<td>7</td>
<td>Hurts or upsets other children without meaning to</td>
</tr>
<tr>
<td>Interests</td>
<td>7</td>
<td>Shows interest in things or activities that most people would find unusual, e.g. traffic lights</td>
</tr>
</tbody>
</table>

3.6.2.3 Repetitive Behavior Scale – Revised (RBS-R)

The RBS-R is a 43-item questionnaire assessing the presence and severity of restricted or repetitive behaviours commonly seen in people with ASDs (317). Items are rated on a Likert scale of severity from 0=‘behaviour does not occur’ to 3=‘behaviour occurs and is a severe problem’. Types of behaviour measured by the RBS-R include stereotyped, self-injurious, compulsive, ritualistic, sameness, and restricted behaviours. Table 3.4 lists example items for these subscales.

The RBS-R was completed by parents/guardians at each visit. Raw scores are totalled for each subscale to give severity scores, while the number of items scored positively for each subscale gives the topography scores, indicating which domain of behaviour is most problematic for the individual. Subscales have high internal reliability (r=.71 to .88) and confirmatory factor analysis showed satisfactory loadings of all items onto their designated subscale (318), while RBS-R severity and topography scores show high inter-rater reliability (r=.88 and r=.82) and test-retest stability (r=.71 and r=.93) (293).

While the psychometric properties of total RBS-R score have not been established, it provides an overall measure of the impact of repetitive and restricted behaviours (319), correlates highly with measures of autism severity and overall functioning (320), and is included in this study for completeness. The RBS-R has been validated for use in children as young as two years of age, and RBS-R scores are correlated with repetitive behaviour dimensions on established autism rating scales such as the Autism Diagnostic Interview – Revised (318).

3.6.3 Safety monitoring and adverse events

At each post-baseline visit, parents/guardians reported on any illness or injury experienced by their child since the previous visit, regardless of any putative relationship to the investigational product. Parents/guardians were also contacted by telephone on a monthly basis between visits to monitor for adverse events. Adverse events were logged both in each participant’s case report form, and on a master log for all participants to monitor for any trends in adverse events. The adverse events checklist, outlined in Table 3.5 and included in full in Appendix C, was completed in
<table>
<thead>
<tr>
<th>RBS-R Subscale (Behaviour type)</th>
<th>No. of items</th>
<th>Example Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereotyped</td>
<td>6</td>
<td>Hand/finger (Flaps hands, wiggles or flicks fingers, claps hands, waves or shakes hand or arm)</td>
</tr>
<tr>
<td>Self-injurious</td>
<td>8</td>
<td>Bites self (Bites hand, wrist, arm, lips or tongue)</td>
</tr>
<tr>
<td>Compulsive</td>
<td>8</td>
<td>Touch/tap (Need to touch, tap, or rub items, surfaces, or people)</td>
</tr>
<tr>
<td>Ritualistic</td>
<td>6</td>
<td>Eating/mealtime (Strongly prefers/insists on eating/drinking only certain things; eats or drinks items in a set order; insists that meal related items are arranged in a certain way)</td>
</tr>
<tr>
<td>Sameness</td>
<td>11</td>
<td>Insists on same routine, household, school or work schedule every day</td>
</tr>
<tr>
<td>Restricted</td>
<td>4</td>
<td>Fascination, preoccupation with one subject or activity (e.g. trains, computers, weather, dinosaurs)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of adverse event</td>
<td>Qualitative description of illness or injury reported</td>
</tr>
<tr>
<td>Date/time event started</td>
<td></td>
</tr>
<tr>
<td>Date/time event ceased</td>
<td></td>
</tr>
<tr>
<td>Reaction intensity</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>Very severe</td>
</tr>
<tr>
<td></td>
<td>Life-threatening</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Relationship to study drug</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Unlikely</td>
</tr>
<tr>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td>Probable</td>
</tr>
<tr>
<td></td>
<td>Certain</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Action taken</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Dose reduced</td>
</tr>
<tr>
<td></td>
<td>Dose interrupted</td>
</tr>
<tr>
<td></td>
<td>Dose increased</td>
</tr>
<tr>
<td></td>
<td>Drug discontinued</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Treatment given (if applicable)</td>
<td>Resolved without sequelae</td>
</tr>
<tr>
<td>Outcome</td>
<td>Resolved with sequelae</td>
</tr>
<tr>
<td></td>
<td>Ongoing</td>
</tr>
</tbody>
</table>
the instance of any illness or injury during the treatment phase of the study and gave a comprehensive picture of the event(s) and any possible relationship to the investigational product.

Any adverse event that required hospitalisation or was considered life threatening was classed as a serious adverse event (SAE). All SAEs were discussed in a meeting of all trial investigators to determine whether participant withdrawal was necessary. Similarly, if the relationship between any adverse event and the trial medication was deemed ‘possible’ or higher, trial investigators discussed the event to assess the need for participant withdrawal and for informing other participants of the event.

A Data Safety Monitoring Board (DSMB) met with trial personnel on at least a 6-monthly basis during the trial. At each meeting, the DSMB reviewed the progress of the trial, including recruitment and participant flow; individual adverse events and aggregate data; and participant withdrawals and protocol violations. The DSMB provided advice on dealing with adverse events and could recommend termination of the trial or changes to the protocol if required.

### 3.6.4 Secondary and other measures

At each post-baseline visit, the interview captured changes or noteworthy events in the participant’s life since the previous visit, including medical problems, changes in interventions or medications, and parents’ impressions of change in their child since baseline. Parents/guardians were also asked whether and why they believed their child was receiving NAC or placebo. The interview form is included in full in Appendix C.

#### 3.6.4.1 Developmental Behaviour Checklist (DBC-P)

The DBC-P was developed by Einfeld and Tonge to assess behavioural and emotional problems in children with intellectual disability (321). It consists of 96 Likert items, rated on a scale from 0=‘not true as far as you know’ to 2=‘very true or often true’. Items form five overlapping subscales: disruptive/antisocial, self-absorbed, communication disturbance, anxiety and social relating. Table 3.6 lists example items from each subscale.
Table 3.6. Subscales of the Developmental Behaviour Checklist and example items

<table>
<thead>
<tr>
<th>DBC-P subscale</th>
<th>No. of items</th>
<th>Example Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disruptive/Antisocial</td>
<td>27</td>
<td>Stubborn, disobedient or uncooperative</td>
</tr>
<tr>
<td>Self-absorbed</td>
<td>31</td>
<td>Aloof, in his/her own world</td>
</tr>
<tr>
<td>Communication disturbance</td>
<td>13</td>
<td>Repeats back what others say like an echo</td>
</tr>
<tr>
<td>Anxiety</td>
<td>9</td>
<td>Upset and distressed over small changes in routine or environment</td>
</tr>
<tr>
<td>Social relating</td>
<td>10</td>
<td>Doesn’t respond to others’ feelings, e.g. shows no response if a family member is crying</td>
</tr>
</tbody>
</table>

Parents/guardians completed the DBC-P at all visits, providing a measure of problematic behaviours throughout the trial. Subscale scores on the DBC-P are derived by adding scores for the relevant items, while an overall score, the Total Behaviour Problem Score (TBPS), is the sum of all items across all subscales. Internal reliability of the DBC-P subscales ranges between $r=.66$ and $r=.91$ (322). The DBC-P has good inter-rater reliability ($r=.52-.77$) and test-retest stability ($r=.76-.89$) (322,323). Finally, DBC-P scores correlate well with equivalent subscales on other measures of problem behaviour, including the Aberrant Behavior Checklist (ABC) and the Child Behavior Checklist (294,322).

Three pilot studies, conducted contemporaneously with the current study, found significant improvements on the Irritability subscale of the ABC (7-9). As there was no direct analogy of the Irritability subscale of the ABC in the measures used in the current study, single items on the DBC-P pertaining to irritability (item 38), impulsivity (item 37) and anxiety (item 85), considered most comparable to the ABC items by the investigators, were examined along with the DBC-P TBPS and subscale scores.

3.6.4.2 Vineland Adaptive Behavior Scales – Second Edition (Vineland-II)

The Vineland-II is a comprehensive measure of adaptive behaviour and daily functioning in individuals from birth to age 90, developed by Sparrow and Cicchetti (295). It comprises five domains: communication, daily living skills, socialisation, motor skills, and maladaptive behaviour. In turn, each domain consists of several subdomains, described in Table 3.7, which form the 14 sections of the Vineland-II.

In all domains except maladaptive behaviour, each item describes an adaptive behaviour or skill, which is rated on frequency of occurrence on a Likert scale. On this scale, 2='usually' (meaning ‘usually or habitually performed without physical help or reminders’), 1='sometimes or partially’ (‘performed sometimes or partially without physical help or reminders’), and 0='never’ (‘never or very seldom performed or never performed without help or reminders’). Within each subdomain, items are arranged according to the age group at which the behaviour or skill described is typically most salient. For instance, under Receptive Communication, for participants aged five years
### Table 3.7. Domains and subdomains of the Vineland Adaptive Behavior Scales

<table>
<thead>
<tr>
<th>Vineland-II domains</th>
<th>Vineland-II subdomains</th>
<th>Total no. of items</th>
<th>Content description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication</td>
<td>Receptive</td>
<td>20</td>
<td>Listening, paying attention, comprehension</td>
</tr>
<tr>
<td></td>
<td>Expressive</td>
<td>54</td>
<td>Use of words and sentences to gather and provide information</td>
</tr>
<tr>
<td></td>
<td>Written</td>
<td>25</td>
<td>Reading and writing</td>
</tr>
<tr>
<td>Daily living skills</td>
<td>Personal</td>
<td>41</td>
<td>Eating, dressing, personal hygiene</td>
</tr>
<tr>
<td></td>
<td>Domestic</td>
<td>24</td>
<td>Performing household tasks</td>
</tr>
<tr>
<td></td>
<td>Community</td>
<td>44</td>
<td>Use of time, money, telephone, computer</td>
</tr>
<tr>
<td>Socialisation</td>
<td>Interpersonal</td>
<td>38</td>
<td>Interaction with others</td>
</tr>
<tr>
<td></td>
<td>relationships</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Play and leisure time</td>
<td>31</td>
<td>Play and use of leisure time</td>
</tr>
<tr>
<td></td>
<td>Coping skills</td>
<td>30</td>
<td>Responsibility and sensitivity to others</td>
</tr>
<tr>
<td>Motor skills</td>
<td>Gross</td>
<td>40</td>
<td>Use of arms and legs for movement and coordination</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>36</td>
<td>Use of hands and fingers to manipulate objects</td>
</tr>
<tr>
<td>Maladaptive behaviour</td>
<td>Internalising</td>
<td>11</td>
<td>Undesirable behaviour that may interfere with adaptive functioning</td>
</tr>
<tr>
<td></td>
<td>Externalising</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

and above, items include ‘Follows instructions in “if-then” form (for example, “If you want to play outside, then put your things away”’); and ‘Listens to a story for at least 15 minutes’. In each subdomain, respondents begin with the first item listed in the child’s age group and proceed until the end of the section.

In the maladaptive behaviour domain, all items are completed for all age groups, rated on a three-point Likert scale consisting of 0=‘never’, 1=‘sometimes’ and 2=‘often’. Examples of items include ‘Is overly anxious or nervous’ for internalising behaviours, and ‘Is physically aggressive (for example, hits, kicks, bites, etc.)’ for externalising behaviours.

The Vineland-II was completed by parents/guardians at 6-month intervals starting from baseline, to assess levels of and changes in adaptive behaviour and overall functioning relative to participants’ age. Scores generated by the Vineland-II include standardised scores for each domain, along with a total Adaptive Behaviour Composite, all of which have a mean of 100 and standard deviation of 15. Subdomain scores are also generated by the Vineland-II, providing detailed information about individuals’ strengths and weaknesses (295).

Detailed information regarding the reliability and validity of the Vineland-II has been gathered. Split-half reliability has been conservatively estimated at between $r=.87$ and $r=.93$ for domain scores for children aged under 11 years, and between $r=.73$ and $r=.93$ for subdomain scores (295). Test-retest reliability with an interval of 2-5 weeks ranged between $r=.73$ and $r=.94$ for children aged 3-13 years. The construct validity of the Vineland-II has been established in factor analyses indicating that items and each subdomain load onto the appropriate domains (295).

3.6.4.3 Clinical and Parent Global Impressions (CGI-S, CGI-I and PGI-I)

The CGI-S was completed at all visits by the clinician as a subjective measure of the clinical severity of each participant’s presentation. It is a 7-point scale requiring the clinician to compare the participant’s severity of illness to their total clinical experience
with other children with autism, ranging from 1='Normal', 2='Minimally ill', 3='Mildly ill’, up to 6='Severely ill' and 7='Very severely ill’.

The PGI-I and CGI-I were completed at each post-baseline visit by the participant’s parent/guardian and the clinician respectively. Both instruments require the rater to assess the change in the participant since baseline on a 7-point scale ranging from 1='Very much improved’ to 4='No change’ to 7='Very much worse’.

Inter-rater reliability was established between the three clinicians conducting trial visits by comparing PGI-S and PGI-I ratings across 25 participants (25.5% of sample).

### 3.7 Statistical considerations

#### 3.7.1 Sample size calculation

The three primary outcomes for this study were continuous measures. For evaluation of intervention efficacy, the outcome of interest is the difference \( D \) between mean change (from baseline to 6 months) in the NAC and placebo groups. The effect size is defined as \( ES = D/\sigma \), where \( \sigma \) is an overall estimate of the standard deviation of change. With a sample of 98 participants (49 per group), the differential change in the main outcomes detectable with 80% power and 2-sided Type I error rate \( \alpha=0.05 \) is 0.57.

#### 3.7.2 Data entry and integrity

Two trial clinicians manually entered raw data into a Microsoft Excel database, where summary scores were also calculated according to each measure’s algorithms. To check for data integrity, ten participant case CRFs (10% of total) were randomly selected for manual double entry by both clinicians. Frequency statistics were also examined across the database, to check for anomalies such as responses outside the possible range. Finally, another ten CRFs were randomly selected for visual comparison against the database by the two clinicians.
3.7.3 Quantitative data analysis

Data analyses were conducted using STATA 13. T-tests and chi-square tests were used to compare baseline characteristics between the intervention and control groups. A modified intention to treat analysis was performed using a linear mixed model approach. The only modification was that participants had to have at least one post-baseline assessment to be included in the analyses. To assess the impact of the intervention on continuous primary and secondary outcomes, time by treatment interactions were examined in a repeated measures split-plot in time ANOVA model. Model parameters were estimated using generalised estimating equations (GEEs) with an exchangeable working correlation matrix to take account of the repeated measures for each participant. The overall p-values for intervention by follow-up interaction were reported. Follow-up by intervention interaction impacts and their 95% confidence intervals were reported. A secondary set of analyses were planned to adjust for any baseline characteristics that were found to be imbalanced between groups to the extent of a 0.25 standard deviation difference in means (quantitative measures) or an odds ratio of 1.5 (binary measures).

Results from the analysis of categorical data are presented as proportions, with 95% confidence interval, and chi-square tests and Fisher’s Exact p-value used to compare group proportions where appropriate. Nonparametric statistics were used when assumptions for parametric methods were violated. All effect sizes (follow-up by intervention effect) were calculated using Cohen's d. All tests of treatment effects were conducted using a two-tailed alpha level of 0.05 and 95% confidence intervals.

3.8 Qualitative data analysis

3.8.1 A mixed-methods clinical trial

This study aimed to supplement the main efficacy study with the use of exploratory qualitative methods. Use of mixed-methods approaches in randomised controlled trials, utilising the subjective reports of participants and/or their carers, may help to confirm the results of quantitative studies. More importantly, qualitative methods may identify unexpected areas of treatment efficacy, outside of symptoms or behaviours examined by
standardised outcome measures (324,325). Mixed-methods approaches, involving a combination of quantitative and qualitative methods, are increasingly being adopted in trials of psychological therapies (326,327); however, their use is rarer in trials of pharmacotherapies.

In their RCT of NAC in adults with schizophrenia, Berk et al. (324) examined participants’ reports and study clinicians’ observations and found significant differences in psychotic symptoms that were not shown in the quantitative analysis, as well as in mood symptoms which were not directly assessed as part of the traditional study design. This led to their subsequent trial of NAC in bipolar disorder (6), where similar investigation of qualitative data found unexpected improvements in nail-biting in the NAC group (328). As well, in the qualitative analysis, signals of improved social interactivity and less social withdrawal provided support for the hypothesis that NAC may be effective in autism (324).

The use of mixed-method approaches in autism treatment trials is promising, given the current lack of established treatments with clearly defined target symptoms, and the poorly understood aetiology of autism. As well, the heterogeneity in clinical presentation of autism means that individuals’ treatment goals may differ from those identified by researchers prior to commencing a study (329). Standardised outcome measures may not provide a full picture of the functional impact of treatment, where small changes in behaviour or symptoms may be subjectively significant to individuals with autism, their families, and their quality of life. Also, domains of change may not overlap with those chosen a priori, and this allows hypothesis generation.

Therefore, this study applied a mixed-methods approach, using the collection and analysis of qualitative data to provide an additional perspective on trial outcomes, and potentially identify unexpected areas of treatment efficacy.

3.8.2 Qualitative methods

In this mixed-methods trial, a Concurrent Embedded strategy was used, involving simultaneous quantitative and qualitative data collection, with the qualitative analysis
supplementing the primary quantitative analysis (330). As noted in Section 3.6, at each visit, trial clinicians wrote summary notes and verbatim quotes where possible, based on parent/guardian reports and direct observation of the participant, for use in the qualitative analysis.

Following the completion of the trial, transcription and analysis of the qualitative data were completed by a research student (331), who was blind to treatment arm throughout the qualitative analysis. Data were entered into Microsoft Excel and hand-coded based on the *DSM-IV-TR* diagnostic criteria for autism, as well as incorporating emerging themes throughout the initial analyses. Individual sentences were classified as either positive reports, including reduction in symptoms or improvement in adaptive or constructive behaviours; or negative reports, including maintenance or worsening of symptoms (331). The frequency of positive and negative reports was counted for each theme across the entire study period.

The purpose of the qualitative analysis was to investigate differences between NAC and placebo groups in the frequency of changes reported in symptoms and behaviour that may not be adequately captured using standardised quantitative scales. Independent samples t-tests were conducted for each theme, using a two-tailed alpha level of 0.05 (331).
Chapter 4. Results

4.1 Participant flow and attrition

In total, 415 interested parents/guardians of prospective participants contacted the research team by phone or email, in response to advertisements and mail-outs. Of these, 136 did not meet inclusion criteria upon initial screening, due to age, diagnosis of a pervasive developmental disorder (PDD) other than autistic disorder (henceforth referred to as ‘autism’), or presence of asthma or epilepsy. A further 177 declined to participate or failed to respond to research team follow-up. Reasons for declining to participate given by parents/guardians included reluctance to risk their child being in the placebo arm and inability to travel to appointments.

A total of 102 children with autism were randomised into the study (n=51 in each treatment arm). Four children were withdrawn prior to completing the baseline appointment, leaving the final intention-to-treat (ITT) sample of 98 participants (n=48 and n=50 in the NAC and placebo groups respectively).

Figure 4.1 displays the overall participant flow through the 6-month study. Seventy-one participants completed all visits (27.6% attrition). For those who did not complete the study, reasons for withdrawal were: protocol violation (n=8); loss to follow-up (n=8); revocation of parents’ consent (n=8); health reasons (n=2); and one withdrawal due to a serious adverse event (SAE). The protocol violations, health issues and the SAE are detailed in the Protocol Adherence, and Safety Monitoring and Adverse Events sections (Sections 4.4 and 4.7) below.

4.2 Sample characteristics

All characteristics described below refer to the ITT sample of 98 randomised participants who attended the baseline appointment. Unless otherwise stated, there were no significant differences on any demographic or baseline symptomatology measure between NAC and placebo groups.
Chapter 4. Results

Figure 4.1. Participant flow and attrition
4.2.1 Birth and medical history

At baseline, the mean age of the sample was 6 years, 4 months (SD=22 months, range=3.1 to 10.1 years). The sample consisted of 79 males (80.6%) and 19 females, consistent with the overall male/female ratio reported in autism. Ninety-five participants (96.9%) were born in Australia, with the remaining three (3.1%) born in Vietnam, United States of America, and United Arab Emirates.

Based on parent/guardian report, approximately 30% of participants’ mothers experienced significant illness or concerns during pregnancy, the most common being infections (5.1%), gestational diabetes (5.1%), pre-eclampsia (4.1%), and early bleeding/labour (5.1%). On average, participants were born at 39.1 weeks gestation (SD=1.9 weeks, range=32-42 weeks), weighing 3.30 kg (SD=0.73 kg, range=1.23-5.24 kg). Fourteen percent of participants required an emergency caesarean due to complications at birth. Other complications at birth included breech (9.2%) or other atypical presentation (3.1%), need for vacuum/forceps delivery (5.1%), jaundice (3.1%), and need for neonatal intensive care (11.2%).

Sixty percent of participants had no reported medical diagnoses or concerns aside from autism. The most frequent concurrent medical issues were recurring ear infections (10.2%), skin conditions such as eczema or dermatitis (9.2%), musculoskeletal issues (7.1%), and gastrointestinal complaints (6.1%). Other medical conditions included allergies (4.1%) and vitamin/mineral deficiency (3.1%).

Birth and medical history characteristics are summarised in Table 4.1 for the NAC and placebo groups.

4.2.2 Family characteristics

The majority of participants (64.3%) and their parents (75.5%) came from an Anglo-Saxon background, with most parents (71.4%) being born in Australia. Other ethnic backgrounds in the study included southern/eastern European (13.8%), Asian (6.1%), Aboriginal Australian (1.0%), and African (1.0%), with 2.0% unknown or undisclosed.
Table 4.1. Birth and medical history characteristics for NAC and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>NAC (n=48)</th>
<th>Placebo (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at baseline (SD)</td>
<td>6 years, 6 months (21.7 months)</td>
<td>6 years, 3 months (23.8 months)</td>
</tr>
<tr>
<td>Age range at baseline</td>
<td>3 years, 4 months-10 years, 1 month</td>
<td>3 years, 1 month-9 years, 7 months</td>
</tr>
<tr>
<td>Male (%)</td>
<td>87.5</td>
<td>74.0</td>
</tr>
<tr>
<td>Born in Australia (%)</td>
<td>95.8</td>
<td>98.0</td>
</tr>
<tr>
<td>Any significant illness/concerns during pregnancy (%)</td>
<td>27.1</td>
<td>32.0</td>
</tr>
<tr>
<td>Any significant complications during birth (%)</td>
<td>39.6</td>
<td>38.0</td>
</tr>
<tr>
<td>Mean birth weight (SD)</td>
<td>3.28 kg (.89 kg)</td>
<td>3.30 kg (.70 kg)</td>
</tr>
<tr>
<td>Other medical diagnoses/concerns (%)</td>
<td>29.6</td>
<td>42.0</td>
</tr>
</tbody>
</table>
Eighteen participants (18.4%) had a language other than English spoken to them at home.

At baseline, most participants (83.7%) lived with both biological parents in one home; 14.3% lived primarily with their mother; two participants, whose mothers were deceased, lived with their father and their grandparents respectively. Almost all participants (94.9%) had at least one biological sibling, most of these (90.8%) living in the same home.

Participants came from a diverse range of socioeconomic backgrounds. Compared to the general population (332), a relatively large proportion of participants’ parents (44.9% of mothers and 28.5% of fathers) had completed a bachelor’s degree or higher. Around a third of parents (35.7% of mothers and 39.8% of fathers) were employed at a professional level or higher. Mean gross household income from all sources was between $60,000 and $80,000 per year. Table 4.2 summarises socioeconomic characteristics for the NAC and placebo groups.

Six families had two children with autism participating in the study (12.2% of participants). Based on parent report, five other participants had at least one non-participant sibling with a diagnosis of autism (5.1%) and one participant had a sibling with another ASD (1.0%). Twenty-one participants had at least one relative with a reported diagnosis of autism (21.4%); 24 had at least one relative with another ASD (24.5%).

4.2.3 Autism symptomatology and baseline presentation

4.2.3.1 Course and early presentation

All participants had received a diagnosis of autism. The mean age that the diagnosis of autism was given was 3 years, 4 months (SD=13.7 months, range=15-90 months), and the mean duration of illness was three years (SD=22.7 months, range=3-87 months). Participants’ parents reported that they first became concerned about their child’s behaviour or development at a mean age of 1 year, 9 months (SD=11.2 months), though
### Table 4.2. Parental socioeconomic background for NAC and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>NAC (n=48)</th>
<th>Placebo (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother’s highest education level (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Year 11 or below</td>
<td>18.8</td>
<td>12.0</td>
</tr>
<tr>
<td>- Year 12</td>
<td>16.7</td>
<td>14.0</td>
</tr>
<tr>
<td>- TAFE/Trade qualification</td>
<td>8.3</td>
<td>4.0</td>
</tr>
<tr>
<td>- Diploma</td>
<td>12.5</td>
<td>22.0</td>
</tr>
<tr>
<td>- Bachelor degree</td>
<td>25.0</td>
<td>36.0</td>
</tr>
<tr>
<td>- Postgraduate</td>
<td>16.7</td>
<td>12.0</td>
</tr>
<tr>
<td><strong>Father’s highest education level (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Year 11 or below</td>
<td>27.1</td>
<td>16.0</td>
</tr>
<tr>
<td>- Year 12</td>
<td>18.8</td>
<td>22.0</td>
</tr>
<tr>
<td>- TAFE/Trade qualification</td>
<td>10.4</td>
<td>16.0</td>
</tr>
<tr>
<td>- Diploma</td>
<td>12.5</td>
<td>10.0</td>
</tr>
<tr>
<td>- Bachelor degree</td>
<td>18.8</td>
<td>24.0</td>
</tr>
<tr>
<td>- Postgraduate</td>
<td>6.3</td>
<td>8.0</td>
</tr>
<tr>
<td><strong>Mother employed outside home at baseline (%)</strong></td>
<td>47.9</td>
<td>62.0</td>
</tr>
<tr>
<td><strong>Father employed outside home at baseline (%)</strong></td>
<td>83.3</td>
<td>88.0</td>
</tr>
<tr>
<td><strong>Mother’s usual occupation – category (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Manager/administrator</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>- Professional</td>
<td>33.3</td>
<td>34.0</td>
</tr>
<tr>
<td>- Associate professional</td>
<td>4.2</td>
<td>6.0</td>
</tr>
<tr>
<td>- Clerical/service</td>
<td>25.1</td>
<td>32.0</td>
</tr>
<tr>
<td><strong>Father’s usual occupation – category (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Manager/administrator</td>
<td>12.5</td>
<td>10.0</td>
</tr>
<tr>
<td>- Professional</td>
<td>27.1</td>
<td>30.0</td>
</tr>
<tr>
<td>- Associate professional</td>
<td>14.6</td>
<td>14.0</td>
</tr>
<tr>
<td>- Tradesperson</td>
<td>16.7</td>
<td>18.0</td>
</tr>
<tr>
<td>- Clerical/service</td>
<td>4.2</td>
<td>12.0</td>
</tr>
<tr>
<td>- Production/transport</td>
<td>2.1</td>
<td>6.0</td>
</tr>
<tr>
<td>- Labourer</td>
<td>6.3</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>Gross household income per year in $000s (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- $0-20</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>- $20-40</td>
<td>17.0</td>
<td>4.0</td>
</tr>
<tr>
<td>- $40-60</td>
<td>21.3</td>
<td>22.0</td>
</tr>
<tr>
<td>- $60-80</td>
<td>17.0</td>
<td>22.0</td>
</tr>
<tr>
<td>- $80-100</td>
<td>21.3</td>
<td>22.0</td>
</tr>
<tr>
<td>- $100-120</td>
<td>8.5</td>
<td>8.0</td>
</tr>
<tr>
<td>- &gt;$120</td>
<td>10.6</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Note.* TAFE, Technical and Further Education.
some parents reported that developmental concerns were present from birth (range=0-72 months).

The most common first concern was abnormal language development (78.6%). Regression in language skills was fairly common, with 40.8% of parents describing a period of normal language development until 12-36 months, followed by a seemingly sudden stopping or reversal of development. Other areas of concern were social interaction (60.2%), such as poor eye contact or lack of response to name; general developmental issues (42.9%), such as difficulty sleeping or feeding; and repetitive behaviours or restricted interests (36.7%).

4.2.3.2 ADOS

Partway through the study, a clinical psychologist began to administer the Autism Diagnostic Observation Schedule (ADOS) at baseline. Subsequently, the ADOS was used to confirm autism diagnosis and provide a clinician-rated measure of symptom severity. All participants had met criteria for autism diagnosis, based on thorough case review prior to baseline by two child psychologists experienced in autism diagnosis and treatment. There was no difference between the 21 participants enrolled before and the 77 participants enrolled after this change on any of the other symptomatology measures administered at baseline.

In total, 77 participants completed the ADOS at baseline, with 28 completing Module 1; 21 completing Module 2; and 28 completing Module 3. Table 4.3 shows baseline ADOS severity and comparison scores for the NAC and placebo groups.

4.2.3.3 Cognitive assessment

Cognitive assessments were conducted at baseline with 49 participants. Current assessment reports, completed in the past two years, were available from other sources for an additional 33 participants. In total, 23 completed the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV), and the other 59 completed the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III).
Table 4.3. Baseline Autism Diagnostic Observation Schedule (ADOS) module completed, severity and comparison scores for NAC and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>NAC (n=38)</th>
<th>Placebo (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number completed (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Module 1</td>
<td>14 (36.8)</td>
<td>14 (35.9)</td>
</tr>
<tr>
<td>- Module 2</td>
<td>9 (23.7)</td>
<td>12 (30.8)</td>
</tr>
<tr>
<td>- Module 3</td>
<td>15 (39.5)</td>
<td>13 (33.3)</td>
</tr>
<tr>
<td>Mean Social Affect score (SD)</td>
<td>15.45 (4.26)</td>
<td>14.67 (4.34)</td>
</tr>
<tr>
<td>Mean Repetitive &amp; Restricted Behaviour score (SD)</td>
<td>2.45 (2.21)</td>
<td>1.82 (1.93)</td>
</tr>
<tr>
<td>Mean ADOS Severity Score (SD)</td>
<td>17.89 (5.44)</td>
<td>16.49 (5.65)</td>
</tr>
<tr>
<td>Mean ADOS Comparison Score (SD)</td>
<td>7.89 (2.31)</td>
<td>7.28 (2.14)</td>
</tr>
</tbody>
</table>
Chapter 4. Results

Table 4.4 displays the cognitive assessment statistics for the NAC and placebo groups. Due to variation in participants’ abilities to complete particular subtests, group statistics for individual WPPSI-III/WISC-IV subtests are not reported here. Valid Full Scale IQ (FSIQ) scores were generated or available for 43 participants, with an age-standardised mean of 73.02 (SD=14.62; range=46-104). Valid Verbal IQ (VIQ) from the WPPSI-III or Verbal Comprehension Index (VCI) from the WISC-IV were available for 52 participants in total, with an age-standardised mean of 71.13 (SD=14.82; range=48-107); 61 participants had valid Performance IQ (PIQ) from the WPPSI-III or Perceptual Reasoning Index (PRI) from the WISC-IV, with an age-standardised mean of 79.77 (SD=18.91; range=45-122).

4.2.3.4 Social Communication Questionnaire (SCQ)

All but six participants (three from each group) completed the SCQ – Lifetime and Current Versions at baseline. For the NAC group, the mean SCQ – Lifetime score was 21.67 (SD=7.01, range=7-34), while the mean SCQ – Current score was 17.63 (SD=6.63, range=5-33). For the placebo group, the mean SCQ – Lifetime score was 21.43 (SD=6.87, range=6-34), while the mean SCQ – Current score was 17.63 (SD=6.63, range=6-28). There was no difference between NAC and placebo groups on either measure.

4.2.4 Schooling and treatments

4.2.4.1 Schooling

Approximately two thirds of participants were attending school at baseline, split across mainstream (26.5%) and special school (33.7%), with a small group attending both (4.1%). Of those attending school, 36.0% had access to a classroom aide. The remaining participants not at school were attending childcare (14.3%) or kindergarten (24.5%). Table 4.5 displays the percentage of participants in the NAC and placebo groups attending school, kindergarten and childcare at baseline. There were no significant differences between groups on type of institution attended at baseline.
Table 4.4. Baseline cognitive assessment completed and valid summary scores reported for NAC and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>NAC (n=40)</th>
<th>Placebo (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number completed (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- WISC-IV</td>
<td>11 (27.5)</td>
<td>12 (28.6)</td>
</tr>
<tr>
<td>- WPPSI-III (old)</td>
<td>23 (57.5)</td>
<td>19 (45.2)</td>
</tr>
<tr>
<td>- WPPSI-III (young)</td>
<td>6 (15.0)</td>
<td>11 (26.2)</td>
</tr>
<tr>
<td>Number with valid FSIQ (%)</td>
<td>22 (55.0)</td>
<td>21 (50.0)</td>
</tr>
<tr>
<td>Mean FSIQ (SD)</td>
<td>74.27 (15.55)</td>
<td>71.71 (13.84)</td>
</tr>
<tr>
<td>Number with valid VIQ/VCI (%)</td>
<td>27 (67.5)</td>
<td>25 (59.5)</td>
</tr>
<tr>
<td>Mean VIQ/VCI (SD)</td>
<td>68.85 (15.40)</td>
<td>73.60 (14.05)</td>
</tr>
<tr>
<td>Number with valid PIQ/PRI (%)</td>
<td>29 (72.5)</td>
<td>32 (76.2)</td>
</tr>
<tr>
<td>Mean PIQ/PRI (SD)</td>
<td>85.38 (17.20)</td>
<td>74.69 (19.20)</td>
</tr>
</tbody>
</table>

Table 4.5. Schooling attended and treatments received for NAC and placebo groups at baseline

<table>
<thead>
<tr>
<th></th>
<th>NAC (n=48)</th>
<th>Placebo (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Schooling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mainstream school (%)</td>
<td>35.5</td>
<td>26.0</td>
</tr>
<tr>
<td>Special school (%)</td>
<td>37.5</td>
<td>38.0</td>
</tr>
<tr>
<td>Access to classroom aide (%) of those attending school</td>
<td>36.4</td>
<td>36.7</td>
</tr>
<tr>
<td>Kindergarten (%)</td>
<td>18.8</td>
<td>30.0</td>
</tr>
<tr>
<td>Childcare (%)</td>
<td>8.4</td>
<td>20.0</td>
</tr>
<tr>
<td><strong>Developmental and behavioural interventions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any developmental/behavioural intervention (%)</td>
<td>79.2</td>
<td>82.0</td>
</tr>
<tr>
<td>Speech therapy (%)</td>
<td>60.4</td>
<td>72.0</td>
</tr>
<tr>
<td>Occupational therapy (%)</td>
<td>45.8</td>
<td>56.0</td>
</tr>
<tr>
<td>Early intervention (%)</td>
<td>20.8</td>
<td>14.0</td>
</tr>
<tr>
<td>ABA (%)</td>
<td>8.3</td>
<td>12.0</td>
</tr>
<tr>
<td>Other intervention (%)</td>
<td>22.9</td>
<td>20.0</td>
</tr>
<tr>
<td><strong>Pharmacotherapies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any medication (%)</td>
<td>47.9</td>
<td>46.0</td>
</tr>
<tr>
<td>Psychotropic medication (%)</td>
<td>20.9</td>
<td>20.0</td>
</tr>
<tr>
<td>Health supplement (%)</td>
<td>20.9</td>
<td>18.0</td>
</tr>
<tr>
<td>Allergy medication (%)</td>
<td>2.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Other (%)</td>
<td>12.6</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Complementary and alternative therapies (CATs)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any CAT (%)</td>
<td>43.7</td>
<td>62.0</td>
</tr>
<tr>
<td>Vitamin/mineral (%)</td>
<td>25.1</td>
<td>26.0</td>
</tr>
<tr>
<td>Modified diet (%)</td>
<td>14.7</td>
<td>24.0</td>
</tr>
<tr>
<td>Manipulation-based (%)</td>
<td>10.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Probiotics (%)</td>
<td>4.2</td>
<td>16.0</td>
</tr>
<tr>
<td>Homeopathy (%)</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Naturopathy (%)</td>
<td>6.3</td>
<td>4.0</td>
</tr>
<tr>
<td>Herbal supplements (%)</td>
<td>0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Note. ABA, Applied Behavioural Analysis.*
4.2.4.2 Developmental and behavioural interventions

All but one participant had received some form of developmental or behavioural intervention in their lifetime (mode=3 (40.8%); range=0-6), with 78.6% of participants currently receiving at least one intervention (mode=2 (34.7%); range=0-5). The number of intervention hours per fortnight at baseline ranged from 0 to 100, with a mean of 6.67 hours.

Table 4.5 displays the percentage of participants in each group receiving developmental or behavioural interventions at baseline. Interventions in the ‘other’ category included social skills (n=11) and floor-time (n=6). There were no significant differences between groups on types of interventions received at baseline. In the NAC group, eight participants commenced new developmental or behavioural interventions during the trial and six ceased existing interventions. In the placebo group, 13 participants commenced new interventions while three ceased existing interventions. There were no significant between-group differences in intervention changes during the trial period.

4.2.4.3 Medications

Medication types received by participants at baseline included psychotropic medication such as melatonin and risperidone (20.4%); health supplements e.g. fish oil (19.3%); anti-allergy medication (4.0%); and other medication e.g. paracetamol (11.2%). Specific psychotropic medications received by participants at baseline were melatonin (12.2%), risperidone (7.1%), fluoxetine (1.0%) and sleeping aids e.g. trimeprazine (2.0%).

Table 4.5 displays the percentage of participants in each group receiving medications at baseline. There were no significant differences between groups on types of medications received at baseline. During the trial, seven participants from the NAC group and 11 participants from the placebo group commenced new medications, which was not a statistically significant difference. No participants in either group ceased taking existing medications during the trial.
4.2.4.4 Complementary and alternative therapies (CATs)

Over half of the participants were receiving some form of CAT at baseline (53.1%), most commonly vitamin/mineral supplements (25.4%); modified diet such as gluten-free/casein-free (GFCF; 19.2%); manipulation-based therapies such as massage and osteopathy (11.1%); and probiotics (10.1%).

Table 4.5 displays the percentage of participants in each group that received CATs at baseline. There were significantly more participants in the placebo group receiving CATs at baseline ($\chi^2(1)=4.04, p<.05$). In the NAC group, two participants commenced new CATs and two participants ceased receiving existing CATs during the trial. Meanwhile, in the placebo group, seven participants commenced new CATs while three participants ceased receiving existing CATs. There were no significant between-group differences in changes to CATs during the trial period.

4.2.5 Characteristics of withdrawn participants

There were no differences on any baseline measure, including the cognitive measures, ADOS and SCQ, between the 71 participants who completed the 6-month trial and the 27 participants who were withdrawn or lost to follow-up. Similarly, those who did not complete the study were not significantly different from those who completed the trial on any demographic variable, except one. The only exception was ethnicity of father ($\chi^2(1)=11.38, p<.01$), with non-completer participants being more likely to have a father from a non-Anglo-Saxon background (OR=5.03, 95% CI=1.89-13.38, $p<.01$).

4.3 Primary outcome measures

There were no significant differences between NAC and placebo treated groups for any of the primary outcome measures: Social Responsiveness Scale (SRS), Children’s Communication Checklist – Second Edition (CCC-2), Repetitive Behavior Scale – Revised (RBS-R), or their subscales, as measured using repeated measures split-plot in time ANOVA analyses and displayed in Tables 4.6 to 4.8. All primary outcomes for baseline to 6 months were compared using the GEE approach as secondary analyses. No
significant intervention effects were found. Cohen’s d effect sizes for secondary analyses were similar to those reported in Tables 4.6 to 4.8.

4.3.1 Social Responsiveness Scale (SRS)

Table 4.6 displays the interaction effects of treatment group by time on the SRS and its subscales. As shown, there was no significant effect of treatment group on SRS total score or subscale scores.

Figure 4.2 shows NAC and placebo groups’ SRS total scores from baseline to 6 months. Both groups’ mean SRS total score significantly decreased from baseline to 6 months (NAC: $t(28)=-3.144$, $p<.01$; Placebo: $t(31)=-4.186$, $p<.001$), indicating an improvement in symptoms over time, but there was no difference between groups on this measure.

Figures 4.3 to 4.7 show all SRS subscales for NAC and placebo from baseline to 6 months. As with total score, the placebo group showed significant improvements on all five SRS subscales over time (Social Awareness: $t(31)=-2.273$, $p<.05$; Social Cognition: $t(31)=-3.268$, $p<.01$; Social Communication: $t(31)=-3.113$, $p<.01$; Social Motivation: $t(31)=-3.263$, $p<.01$; Autistic Mannerisms: $t(31)=-3.229$, $p<.01$). The NAC group showed significant improvements only on the Social Communication ($t(28)=-2.664$, $p<.05$), Social Motivation ($t(28)=-3.165$, $p<.01$) and Autistic Mannerisms ($t(28)=-2.926$, $p<.01$) subscales. There were no significant differences between groups on any subscale.
### Table 4.6. Intervention by time analysis results for Social Responsiveness Scale: NAC vs placebo

<table>
<thead>
<tr>
<th></th>
<th>Baseline to 1 month (Interaction effect (95% CI))</th>
<th>Baseline to 3 months (Interaction effect (95% CI))</th>
<th>Baseline to 6 months (Interaction effect (95% CI))</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Score</strong></td>
<td>-0.2 (-7.3, 7.6)</td>
<td>-4.2 (-12.1, 3.7)</td>
<td>0.9 (-8.0, 9.7)</td>
<td>0.61</td>
</tr>
<tr>
<td>Social Awareness</td>
<td>-0.15 (-1.5, 1.2)</td>
<td>-0.6 (-1.9, 0.7)</td>
<td>-0.4 (-1.9, 1.1)</td>
<td>0.82</td>
</tr>
<tr>
<td>Social Cognition</td>
<td>-0.2 (-2.0, 1.6)</td>
<td>0.0 (-1.7, 1.7)</td>
<td>-0.6 (-2.8, 1.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Social Communication</td>
<td>-1.6 (-4.7, 1.4)</td>
<td>-2.1 (-5.6, 1.4)</td>
<td>-0.5 (-4.0, 3.0)</td>
<td>0.60</td>
</tr>
<tr>
<td>Social Motivation</td>
<td>0.2 (-1.4, 1.9)</td>
<td>0.5 (-1.3, 2.4)</td>
<td>-0.3 (-2.4, 1.8)</td>
<td>0.82</td>
</tr>
<tr>
<td>Autistic Mannerisms</td>
<td>1.0 (-0.8, 2.9)</td>
<td>-1.0 (-2.9, 0.9)</td>
<td>0.3 (-1.7, 2.3)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* Overall intervention by follow-up p-value

**Note.** Follow-up vs. baseline by intervention group interaction uses placebo at baseline as reference group.
Figure 4.2. Total scores on the Social Responsiveness Scale: NAC vs placebo
Figure 4.3. Social Awareness subscale scores on Social Responsiveness Scale: NAC vs placebo
Figure 4.4. Social Cognition subscale scores on Social Responsiveness Scale: NAC vs placebo
Figure 4.5. Social Communication subscale scores on Social Responsiveness Scale: NAC vs placebo
Figure 4.6. Social Motivation subscale scores on Social Responsiveness Scale: NAC vs placebo
Figure 4.7. Autistic Mannerisms subscale scores on Social Responsiveness Scale: NAC vs placebo
4.3.2 *Children’s Communication Checklist (CCC-2)*

Table 4.7 displays the interaction effects of treatment group by time on summary scores on the CCC-2. As shown, there was no significant effect of treatment group on General Communication Composite (GCC) or the Social Interaction Deviance Composite (SIDC) scores.

Figure 4.8 shows GCC scores for NAC and placebo on the CCC-2 from baseline to 6 months. Neither group showed significant changes in GCC over time, nor were there significant difference between groups on this measure.

Figure 4.9 shows SIDC scores for NAC and placebo on the CCC-2 from baseline to 6 months. The NAC group did not show any significant change over time on this measure, while the placebo group’s mean SIDC score significantly decreased from baseline to 6 months ($t(16)=-3.388, p<.01$), broadly indicating an improvement in pragmatic language skills without a commensurate improvement in social language skills. There was no significant difference in SIDC scores between the two groups.
Table 4.7. Intervention by time analysis results for Children’s Communication Checklist: NAC vs placebo

<table>
<thead>
<tr>
<th></th>
<th>Baseline to 1 month</th>
<th>Baseline to 3 months</th>
<th>Baseline to 6 months</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interaction effect (95% CI)</td>
<td>Effect size</td>
<td>Interaction effect (95% CI)</td>
<td>Effect size</td>
</tr>
<tr>
<td>General Communication Composite</td>
<td>0.7 (-4.0, 5.4)</td>
<td>0.09</td>
<td>-1.8 (-9.0, 5.3)</td>
<td>-0.15</td>
</tr>
<tr>
<td>Social Interaction Deviance Composite</td>
<td>-1.5 (-4.8, 1.7)</td>
<td>-0.17</td>
<td>1.0 (-3.2, 5.2)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Overall intervention by follow-up p-value
Note: Follow-up vs. baseline by intervention group interaction uses placebo at baseline as reference group.
Figure 4.8. General Communication Composite scores on Children's Communication Checklist: NAC vs placebo
Figure 4.9. Social Interaction Deviance Composite scores on Children's Communication Checklist: NAC vs placebo
4.3.3 Repetitive Behavior Scale – Revised (RBS-R)

Table 4.8 displays the interaction effects of treatment group by time on the RBS-R and its subscales. As shown, there was no significant effect of treatment group on RBS-R total score or subscale scores.

Figure 4.10 shows NAC and placebo groups’ RBS-R total scores from baseline to 6 months. The placebo group showed a significant improvement in repetitive behaviours over time, as measured by RBS-R total score ($t(31)=-2.110, p<.05$), while the NAC group did not show any significant change over time. There was no significant difference between groups on RBS-R total score.

Figures 4.11 to 4.16 show all RBS-R subscales for NAC and placebo from baseline to 6 months. As with RBS-R total scores, the NAC group did not show any significant changes over time on any of the six RBS-R subscales. The placebo group showed significant improvements only on the Ritualistic Behaviours subscale ($t(31)=-3.022, p<.01$). There were no significant differences between groups on any of the RBS-R subscales.
**Table 4.8. Intervention by time analysis results for Repetitive Behavior Scale: NAC vs placebo**

<table>
<thead>
<tr>
<th></th>
<th>Baseline to 1 month</th>
<th>Baseline to 3 months</th>
<th>Baseline to 6 months</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interaction effect (95% CI)</td>
<td>Effect size</td>
<td>Interaction effect (95% CI)</td>
<td>Effect size</td>
</tr>
<tr>
<td>Total Score</td>
<td>-0.2 (-5.1, 4.7)</td>
<td>-0.10</td>
<td>1.5 (-4.0, 6.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>Stereotyped Behaviours</td>
<td>0.3 (-0.8, 1.4)</td>
<td>0.07</td>
<td>0.5 (-0.5, 1.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>Self-injurious Behaviours</td>
<td>0.1 (-0.8, 1.1)</td>
<td>0.04</td>
<td>-0.2 (-1.3, 0.8)</td>
<td>-0.04</td>
</tr>
<tr>
<td>Compulsive Behaviours</td>
<td>0.0 (-1.3, 1.2)</td>
<td>-0.05</td>
<td>0.2 (-1.0, 1.4)</td>
<td>0.17</td>
</tr>
<tr>
<td>Ritualistic Behaviours</td>
<td>-0.2 (-1.3, 0.8)</td>
<td>0.02</td>
<td>0.7 (-0.5, 1.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Sameness Behaviours</td>
<td>-0.6 (-2.1, 1.1)</td>
<td>-0.17</td>
<td>0.5 (-1.3, 2.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>Restricted Behaviours</td>
<td>0.3 (-0.8, 1.4)</td>
<td>0.07</td>
<td>0.3 (-0.8, 1.5)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Overall intervention by follow-up p-value

Note: Follow-up vs. baseline by intervention group interaction uses placebo at baseline as reference group.
Figure 4.10. Total scores on the Repetitive Behavior Scale: NAC vs placebo
Figure 4.11. Stereotyped Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
Figure 4.12. Self-injurious Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
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Figure 4.13. Compulsive Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
Figure 4.14. Ritualistic Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
Figure 4.15. Sameness Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
Figure 4.16. Restricted Behaviours subscale scores on Repetitive Behavior Scale: NAC vs placebo
4.4 Safety monitoring and adverse events

Overall, NAC was well tolerated by participants, with no significant difference in the number and severity of adverse events between NAC and placebo at months 1, 3 and 6. Table 4.9 shows the number and type of adverse events by group. Gastrointestinal symptoms, including diarrhoea, vomiting and stomach cramps, and colds/cold-like symptoms were the most frequently experienced adverse events in both groups.

There were two serious adverse events (SAEs) involving the hospitalisation of participants. One participant in the NAC group was hospitalised for dental surgery, apparently unrelated to the trial, and was able to continue participation without interruption. A participant from the placebo group required hospitalisation for treatment of a bowel obstruction, and was subsequently withdrawn from the study. Case review by the investigators indicated that this was unlikely to be related to the trial medication, as the participant had experienced ongoing bowel issues prior to study commencement, and symptoms continued post-cessation of the trial medication. Both SAEs were reported to relevant ethics committees and the Data Safety Monitoring Board (DSMB).

Two participants, one from each group, were withdrawn by their parents due to adverse events. One participant in the placebo group experienced a fever shortly after study commencement, and one participant in the NAC group experienced worsening of eczema symptoms. In both cases, review by the investigators and DSMB suggested that a relationship to trial medication was possible; close monitoring for similar future events was implemented but did not result in any further withdrawals for these reasons.

One participant in the NAC group experienced worsening of pre-existing asthma and was withdrawn by investigators shortly after the 3-month visit. Asthma was an exclusion criterion for this study as NAC has previously been shown to aggravate symptoms; however, the participant’s parents had not reported the asthma to investigators at screening. This was therefore treated as a protocol violation and the participant was subsequently withdrawn. The participant’s asthma symptoms improved upon cessation of the trial medication.
Table 4.9. Incidence of participants experiencing non-serious adverse events for NAC and placebo groups

<table>
<thead>
<tr>
<th>Type of adverse event</th>
<th>NAC (n=48)</th>
<th>Placebo (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colds/cold-like symptoms (%)</td>
<td>7 (7.1)</td>
<td>11 (11.2)</td>
</tr>
<tr>
<td>Gastrointestinal symptoms (%)</td>
<td>6 (6.1)</td>
<td>6 (6.1)</td>
</tr>
<tr>
<td>Ear infections (%)</td>
<td>4 (4.1)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Skin problems (%)</td>
<td>2 (2.0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Cysts (%)</td>
<td>1 (1.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Tonsillitis (%)</td>
<td>1 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>2 (2.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Aggravation of pre-existing asthma (%)</td>
<td>1 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract infection (pre-existing issue) (%)</td>
<td>0</td>
<td>1 (1.0)</td>
</tr>
</tbody>
</table>
4.5 Secondary outcome measures

There was no difference between NAC and placebo groups on any of the secondary outcomes, the Developmental Behaviour Checklist – Primary Carer Version (DBC-P); Vineland Adaptive Behavior Scales – Second Edition (Vineland-II); and the global impression scales, the Parent Global Impression – Improvement scale (PGI-I), Clinical Global Impression – Improvement scale (CGI-I), and Clinical Global Impression – Severity scale (CGI-S). Similarly, the NAC and placebo groups did not differ on any Vineland-II domain scores, DBC-P subscales, or the individual DBC-P items that were selected as comparable to the Aberrant Behavior Checklist – Irritability subscale items. All secondary outcomes for baseline to 6 months were compared using the GEE approach as secondary analyses. No significant intervention effects were found. Cohen’s d effect sizes for secondary analyses were similar to those reported in Tables 4.10 and 4.11.

Tables 4.10 and 4.11 display summary statistics and interaction effects of treatment group by time on the DBC-P, global impression scales, and Vineland-II, as well as subscales of the DBC-P and Vineland-II. As shown, there was no significant effect of treatment group on any secondary outcome measure in this study.

Post-hoc examination of CGI-I scores was also conducted, to investigate whether a subgroup of participants may have responded to NAC, with clinical response defined by a CGI-I score of 1=‘very much improved’ or 2=‘much improved’ at 6 months. There was no significant difference in the rate of clinical response between the NAC and placebo groups.
<table>
<thead>
<tr>
<th>Summary descriptive: Mean (SD)</th>
<th>Intervention by follow-up interaction&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>p-value&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>NAC</td>
<td>PBO</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Developmental Behaviour Checklist</strong></td>
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<tr>
<td>TBPS</td>
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<td></td>
<td>(23.2)</td>
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<td>Disruptive</td>
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<td>(7.8)</td>
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<tr>
<td>Self-absorbed</td>
<td>19.7</td>
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<tr>
<td></td>
<td>(9.7)</td>
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<tr>
<td>Communication</td>
<td>7.8</td>
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<tr>
<td>disturbance</td>
<td>(4.5)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>(3.4)</td>
</tr>
<tr>
<td>Social relating</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
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<td>Impulsive item</td>
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<td>(item 37)</td>
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<tr>
<td>Irritable item</td>
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<td>(item 38)</td>
<td>(0.6)</td>
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<td>Anxiety item</td>
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<td>(item 85)</td>
<td>(0.7)</td>
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<td><strong>Global impressions scales</strong></td>
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<td>CGI-S</td>
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<td></td>
<td>(0.52)</td>
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<td>PGI-I</td>
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<td>(0.71)</td>
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</table>

* Overall intervention by follow-up p-value
# Follow-up vs. baseline by intervention group interaction with placebo at baseline as reference group

### Table 4.11. Intervention by time analysis results for Vineland Adaptive Behaviors Scale: NAC vs placebo

<table>
<thead>
<tr>
<th>Summary descriptive: Mean (SD)</th>
<th>Intervention by follow-up interaction#</th>
<th>Baseline to 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>Adaptive Behaviour Composite</td>
<td>NAC</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>70.73</td>
<td>69.86</td>
</tr>
<tr>
<td></td>
<td>(14.75)</td>
<td>(12.46)</td>
</tr>
<tr>
<td>Communication</td>
<td>NAC</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>71.33</td>
<td>69.86</td>
</tr>
<tr>
<td></td>
<td>(17.33)</td>
<td>(15.93)</td>
</tr>
<tr>
<td>Daily living skills</td>
<td>NAC</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>73.40</td>
<td>72.59</td>
</tr>
<tr>
<td></td>
<td>(18.72)</td>
<td>(15.89)</td>
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<tr>
<td>Socialization</td>
<td>NAC</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>71.58</td>
<td>72.18</td>
</tr>
<tr>
<td></td>
<td>(15.56)</td>
<td>(12.84)</td>
</tr>
</tbody>
</table>

* Intervention by follow-up p-value

# Follow-up vs. baseline by intervention group interaction with placebo at baseline as reference group

†Mean difference
4.6 Qualitative analysis

Qualitative data were available for 42 participants from the NAC group and 43 participants from the placebo group. Themes identified are detailed in the results supplied by Craven (331) and roughly aligned with the three core symptom clusters of autism: social interaction (e.g. ‘Making friends, interest in peers’); communication (e.g. ‘Verbal communication’); and repetitive or stereotyped behaviours or interests (e.g. ‘Insistence on sameness/routine/ inflexibility’). Themes relating to general behaviour were also identified, such as ‘Calmness’, ‘Concentration’ and ‘Sleep’ (331).

Each individual report was coded as positive or negative as described in Section 3.8.2. The mean frequency of positive and negative themes reported in the NAC and placebo groups at post-baseline visits are displayed in Table 4.12.

A series of independent samples t-tests was conducted by an Honours candidate blind to treatment allocation, to compare the frequency of positive and negative parent reports of participant behaviour across the study period. Significant differences were found on the themes ‘Calmness’ ($t(59)=2.82, p<.01$) and ‘Verbal communication’ ($t(83)=2.26, p<.05$), with parents in the NAC group more frequently reporting improvements in these areas than parents in the placebo group. There were no significant differences found on any other themes (331).

4.7 Protocol adherence

Adherence to the study protocol could not be gauged based on capsule return, as anecdotal reports indicated that many parents/guardians experienced difficulties delivering capsules to participants. This sometimes resulted in doses being missed despite capsules being disposed of, or multiple capsules being used in a single day, which cannot be accounted for by a simple count of capsules returned. Possible solutions to this problem are proposed in the Discussion (Section 5.3.3).

Eight participants were withdrawn due to protocol violations, five from the NAC group. One protocol violation, failure to disclose pre-existing asthma, was previously described in the Safety Monitoring and Adverse Events section (Section 4.4). Two participants
Table 4.12. Mean (SD) frequency of qualitative themes reported in NAC and placebo groups

<table>
<thead>
<tr>
<th>Theme</th>
<th>NAC (n=42)</th>
<th>Placebo (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive reports</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conversation and social approach</td>
<td>0.76 (0.98)</td>
<td>0.49 (0.67)</td>
</tr>
<tr>
<td>Share interests, emotions, affect</td>
<td>0.14 (0.35)</td>
<td>0.19 (0.39)</td>
</tr>
<tr>
<td>Initiate or respond socially</td>
<td>0.43 (0.70)</td>
<td>0.35 (0.57)</td>
</tr>
<tr>
<td>Verbal communication</td>
<td>1.00 (0.99)</td>
<td>0.58 (0.70)</td>
</tr>
<tr>
<td>Non-verbal communication</td>
<td>0.07 (0.26)</td>
<td>0.70 (0.26)</td>
</tr>
<tr>
<td>Eye contact, body language, gestures, facial expressions</td>
<td>0.17 (0.44)</td>
<td>0.14 (0.51)</td>
</tr>
<tr>
<td>Social relationships/adjusting behaviours</td>
<td>0.36 (0.53)</td>
<td>0.35 (0.61)</td>
</tr>
<tr>
<td>Sharing imaginative play</td>
<td>0.07 (0.26)</td>
<td>0.02 (0.15)</td>
</tr>
<tr>
<td>Making friends, interest in peers</td>
<td>0.36 (0.53)</td>
<td>0.28 (0.50)</td>
</tr>
<tr>
<td>Stereotyped repetitive behaviours</td>
<td>0.07 (0.26)</td>
<td>0.05 (0.21)</td>
</tr>
<tr>
<td>Self-injurious behaviours</td>
<td>0.02 (0.15)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Insistence on sameness/routine/inflexibility</td>
<td>0.14 (0.35)</td>
<td>0.47 (0.21)</td>
</tr>
<tr>
<td>Highly restricted fixated interests</td>
<td>0.00 (0.00)</td>
<td>0.23 (0.15)</td>
</tr>
<tr>
<td>Hyper/hypo sensitivity to sensory input</td>
<td>0.09 (0.30)</td>
<td>0.47 (0.21)</td>
</tr>
<tr>
<td>Compliance vs. defiance</td>
<td>0.19 (0.40)</td>
<td>0.07 (0.26)</td>
</tr>
<tr>
<td>Calmness</td>
<td>0.70 (0.90)</td>
<td>0.26 (0.44)</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.19 (0.51)</td>
<td>0.19 (0.39)</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.02 (0.15)</td>
<td>0.70 (0.26)</td>
</tr>
<tr>
<td>Behaviour (general – unspecified)</td>
<td>0.05 (0.22)</td>
<td>0.12 (0.32)</td>
</tr>
<tr>
<td>Other</td>
<td>0.43 (0.67)</td>
<td>0.35 (0.72)</td>
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<tr>
<td>Social and emotional cluster</td>
<td>1.33 (1.56)</td>
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<tr>
<td>Communicative behaviour cluster</td>
<td>1.24 (1.28)</td>
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<td>Relationship cluster</td>
<td>0.79 (0.84)</td>
<td>0.65 (1.02)</td>
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<tr>
<td>Repetitive and injurious behaviour cluster</td>
<td>0.95 (0.30)</td>
<td>0.05 (0.21)</td>
</tr>
<tr>
<td><strong>Negative reports</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conversation and social approach</td>
<td>0.05 (0.21)</td>
<td>0.02 (0.15)</td>
</tr>
<tr>
<td>Share interests, emotions, affect</td>
<td>0.95 (0.30)</td>
<td>0.23 (0.15)</td>
</tr>
<tr>
<td>Initiate or respond socially</td>
<td>0.02 (0.15)</td>
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<td>0.09 (0.30)</td>
<td>0.09 (0.29)</td>
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<tr>
<td>Making friends, interest in peers</td>
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</tr>
<tr>
<td>Stereotyped repetitive behaviours</td>
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<td>0.19 (0.45)</td>
</tr>
<tr>
<td>Self-injurious behaviours</td>
<td>0.71 (0.34)</td>
<td>0.47 (0.21)</td>
</tr>
<tr>
<td>Insistence on sameness/routine/inflexibility</td>
<td>0.12 (0.40)</td>
<td>0.12 (0.32)</td>
</tr>
<tr>
<td>Highly restricted fixated interests</td>
<td>0.00 (0.00)</td>
<td>0.47 (0.21)</td>
</tr>
<tr>
<td>Hyper/hypo sensitivity to sensory input</td>
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</tr>
<tr>
<td>Compliance vs. defiance</td>
<td>0.19 (0.50)</td>
<td>0.12 (0.32)</td>
</tr>
<tr>
<td>Calmness</td>
<td>0.51 (0.75)</td>
<td>0.48 (0.67)</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.02 (0.15)</td>
<td>0.70 (0.26)</td>
</tr>
<tr>
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<td>0.24 (0.15)</td>
<td>0.23 (0.15)</td>
</tr>
<tr>
<td>Other</td>
<td>0.26 (0.63)</td>
<td>0.70 (0.26)</td>
</tr>
</tbody>
</table>

Source: Craven (331).
from the NAC group were withdrawn due to complete refusal to take the trial medication, compared to zero from the placebo group. The remaining five participants met the withdrawal criterion of missing at least seven consecutive days of trial medication. Reasons reported by parents for missing doses largely revolved around lifestyle issues, e.g. a participant in the placebo group and his family went overseas and forgot to bring the trial medication with them.

4.8 Summary

The trial was conducted in a sample of 98 participants with autism. NAC (500 mg daily) did not differ from placebo in terms of safety and tolerability. There was no significant difference between NAC and placebo on any primary or secondary quantitative outcome measure. The only significant differences were found in the qualitative analysis on calmness and verbal communication, with parents in the NAC group more frequently reporting improvements in these areas than parents in the placebo group.
Chapter 5. Discussion

This double-blind, randomised, placebo-controlled trial of 500 mg daily oral N-acetyl cysteine (NAC) in children with autistic disorder (henceforth referred to as ‘autism’) failed to find any significant effect on primary or secondary outcome measures. This study had the largest sample and longest treatment duration (6 months) of any NAC trial in autism to date, but did not replicate the findings of smaller pilot trials (7-9). There was a signal in the qualitative data, taken from parent reports, suggesting greater rates of improved calmness and verbal communication in NAC than placebo treated individuals. This may be consistent with the findings of decreased irritability in the extant literature, but post hoc findings need to be interpreted with great caution.

This chapter summarises the findings of the study, reviews its strengths and limitations, and comments on implications and possibilities for future research.

5.1 Summary of findings

5.1.1 Sample characteristics

The intention-to-treat sample comprised 98 children with autism aged 3-10 years (mean age=6 years, 4 months, SD=22 months), recruited from the community using a wide range of methods. The sample may be considered reasonably representative, with a gender ratio (4:1) similar to that in the population of children with autism (40), and comprising participants from diverse socioeconomic backgrounds.

Inclusion was based on DSM-IV-TR criteria for autistic disorder (2), as the DSM-5 had not been published at the time the study commenced. A recent systematic review found that between 75 and 100% of individuals diagnosed with DSM-IV-TR autistic disorder would retain their diagnosis if assessed under DSM-5 criteria (333). This suggests that the majority of participants in the current study would likely remain eligible were the study conducted again using DSM-5 criteria; however, this cannot be confirmed at this stage without conducting new assessments or case reviews.
There were no inclusion criteria regarding severity of autism symptomatology, provided diagnosis could be confirmed from case review and/or baseline administration of the Autism Diagnostic Observation Schedule (ADOS). The wide age range of 3-10 years, as well as the underlying heterogeneity of autism, meant that many participants were at different stages of development and traditional intervention exposure, and presented with a variety of areas of strength and difficulty. As a result, participants had a wide range of baseline scores on symptom severity, language skills, and adaptive functioning. Participants had received between zero and six different types of interventions prior to baseline, for anywhere up to 100 hours per fortnight.

5.1.2 Primary and secondary outcomes

Primary outcomes included the core autism symptom domains of social interaction, communication and repetitive behaviours, as measured by the Social Responsiveness Scale (SRS), Children’s Communication Checklist – Second Edition (CCC-2), Repetitive Behavior Scale – Revised (RBS-R), respectively, and their subscales. Secondary outcomes included adaptive behaviours, as measured by the Vineland Adaptive Behavior Scale – Second Edition (Vineland-II), problem behaviours, as measured by the Developmental Behaviour Checklist – Primary Carer Version (DBC-P), and Parent/Clinical Global Impression (PGI and CGI – Severity and Improvement scales). There were no significant treatment effects found on any outcome measure, even prior to Bonferroni correction for multiple testing.

The quantitative findings are in contrast to three previously published pilot trials of NAC in children with autism (7-9), all of which found significant improvements on the Irritability subscale of the Aberrant Behavior Checklist (ABC). One study also found that NAC improved social cognition and autistic mannerisms on the SRS, and decreased stereotypies on the RBS-R (7).

There are several possible reasons that this study did not replicate the results of the previous trials. A possible reason is the difference in dosages used, which is elucidated further in section 5.3.1. Additionally, the trials by Hardan et al. (7) and Nikoo et al. (9) each had inclusion/exclusion criteria regarding baseline symptom severity: scores above
4 on the CGI – Severity (CGI-S) scale, and scores above 12 on the ABC – Irritability subscale, respectively. Each of the three pilot studies with positive findings seemed to recruit only participants from outpatient paediatric psychiatry clinics (7-9), though this is difficult to confirm from the published reports. In contrast, the current study recruited from the wider community and did not have any criteria for baseline severity, potentially resulting in the inclusion of milder cases less likely to show significant improvements on outcome measures.

This is demonstrated by the range of CGI-S scores in the current study, 2 to 7, compared to the range of 4 to 6 in the Hardan et al. study (7). However, inspection of baseline summary scores suggests that the sample in the current study was similar to the sample in the Hardan et al. study (7) on mean CGI-S score, and mean and range of SRS total score. It is more difficult to compare the sample in the current study to that in the Nikoo et al. study (9), as well as the Ghanizadeh et al. study (8), as the authors only report ABC scores, which was not used in the current study.

5.1.3 Qualitative findings

In addition to the quantitative measures, parent/guardian reports and clinicians’ observations were recorded in participant case report forms over the course of the study, and subsequently analysed, blind to treatment allocation, for emergent themes. Compared to the placebo group, there were significantly more frequent reports of increased calmness in children in the NAC group (331). ‘Calmness’ in this study referred to reductions in aggression, tantrums, anxiety, agitation and frustration, including descriptions such as “more chilled”, “settling better”, and “fewer meltdowns”. This may be considered to align with the findings of decreased irritability in the other NAC trials (7-9,331), even though this was not borne out in the quantitative measure (DBC) in the current study.

Significantly more frequent reports of improvements to verbal communication were also found in the NAC group. These findings were relatively unprecedented, given that none of the previous pilot studies of NAC in autism included a measure of verbal communication. A single case report of a child with autism in 2012 did suggest that
NAC may improve verbal communication, based on parental evaluation using a visual analogue scale (289), but this was not explored further in the subsequent RCT by the same group (8).

It is worth considering that reports of improved verbal communication in children with autism may represent increases in social interest or motivation, rather than specific advances in vocabulary or the ability to speak. There is some precedent for NAC improving social interaction, as in a similar qualitative analysis in a mixed-methods RCT of NAC in adults with schizophrenia (324). It is difficult to speculate on the underlying mechanism of efficacy in the current study, however, without significant findings on any measure of communication skills or social interaction. Because of the risks of type I error, qualitative data needs to be seen as hypothesis generating, but the veracity of these data is supported by the fact that they dovetail with the findings of the previous studies.

Since these changes were not found on the quantitative outcome measures, this study demonstrates the potential of mixed-methods clinical trials in identifying areas of treatment efficacy in trials of novel therapies beyond those measured by traditional means (325).

### 5.2 A mixed-methods clinical trial

The current study used both quantitative measures and exploratory qualitative analysis to investigate the efficacy of NAC in children with autism. While mixed-methods approaches are becoming more common in some areas of research, such as trials of psychological therapies, such approaches are still rare in trials of pharmacotherapies. Berk et al. demonstrated the potential of mixed-methods clinical trials in their studies of NAC in bipolar disorder and schizophrenia (6,324,328). Indeed, similar to the current study, those authors revealed further significant outcomes based on the qualitative data. As discussed in Section 3.8.1, the combination of quantitative and qualitative methods could be particularly useful in studying novel therapies with unclear clinical profiles such as autism treatment trials, given the current lack of established treatments with clearly defined target symptoms, and the poorly understood aetiology of autism. Target
symptoms identified by researchers prior to commencing a study, and hence the outcome measures selected, may not align with participants’ and their families’ treatment goals, especially in such a heterogeneous disorder (329).

While changes in calmness/irritability and verbal communication did not reach levels of statistical significance on the quantitative measures (DBC and CCC-2) in this study, parents/guardians and clinicians observing participants in the NAC group reported improvements in these areas that they felt were noteworthy, significantly more often than those in the placebo group. The non-significant results on the quantitative measures of calmness/irritability and verbal communication are in clear contrast with the frequency of qualitative reports of improvement by parents/guardians and clinicians.

A possible reason for this contrast is that the measures used may not be sensitive enough to tap subjectively noticeable improvements in behaviour or symptoms. For example, the response options on the CCC-2 for frequency of communicative behaviours are: 0=‘less than once a week (or never)’; 1=‘several times a week (or sometimes)’; 2=‘1-2 times a day (or often)’; and 3=‘several times (more than twice) a day (or always)’. For children at the more severe end of the spectrum, a transition from exhibiting a communicative behaviour ‘never’ to ‘less than once a week’ may be of major importance to them and their families, but would be rated identically on the CCC-2. A traditional clinical trial design alone would miss this distinction.

The findings of this study may be an illustration of perhaps the most important advantage of mixed-methods clinical trials. Small changes in behaviour or symptoms may be subjectively significant to individuals with autism and their families, without reaching significance levels on standardised outcome measures. Without seeking and analysing the first-person reports of participants and their carers, potentially important areas of treatment efficacy may be missed.

5.3 **Strengths and limitations of the current study**

Limitations of this study included its heterogeneous sample, relatively high attrition rate, problems with protocol adherence, reliance on parent/guardian report, and absence
of biomarker tests. The comparatively low, fixed dose of NAC used, 500 mg daily, also may have contributed to the null quantitative results. Reasons for and possible implications of these limitations, as well as some of the strengths of the study, are discussed below.

5.3.1 Dosage

This study used a much lower dose of NAC than the previously reported trials, where doses ranged between 600 and 2700 mg daily in two or three equally divided doses (7-10). The fixed dose of 500 mg once daily was selected in this study because of concerns about excessively dosing the youngest and smallest participants, and the fact that the safety and tolerability of NAC had not yet been tested in children for an extended duration (6 months). As a proof of concept trial, it was also decided that a fixed dose would be the most feasible approach. Previous NAC trials in adults by Berk et al. used doses of 2000 mg daily (5,6). At the time of obtaining ethical approval, prior to the other pilot trials being published, it was anticipated that the target age group would be younger (3-6 years) than that included in the final study (3-10 years). This dose was therefore deemed an acceptably safe choice by the study investigators and Barwon Health Human Research Ethics Committee. Indeed, the dosage of NAC used in this study was found to be as safe and tolerable as placebo. Unfortunately, the comparatively low dose may be a reason that this study did not replicate the results of the previous trials of NAC in children with autism.

5.3.2 Sample and attrition

At baseline, there was a wide range of symptom severity and traditional intervention exposure among participants. Recruitment from the community and the absence of inclusion criteria for severity meant that relatively mild cases were included in the study. While this heterogeneity may be representative of the broader population of children with autism, it may have made it difficult to detect changes across aggregate data. Future studies may attempt to subtype autism, and could focus on baseline measures of oxidative stress as a predictive biomarker for antioxidant treatment response.
The rates of withdrawal (19.4%) and loss to follow-up (8.2%), while broadly concordant with the existing literature (334), were relatively high compared to the previous pilot NAC studies in autism, which ranged from 12.2% (7) to 22.5% (8). The higher attrition rates in the current study were likely related to its relatively long duration – six months, compared to 8-12 weeks for the previous NAC studies – as well as the time commitment involved in completing trial appointments and questionnaires. Generally, participants’ families were highly motivated to take part, given that they had responded to advertisements in the community rather than being actively recruited. As well, attempts were made to limit the burden of participation, such as conducting between-visit telephone calls instead of increasing the frequency of study visits. Nonetheless, the time commitment may have been onerous for some participants, particularly when considering the daily task of administering the trial medication.

However, it is worth noting that there was no difference between participants who completed the trial and those who were withdrawn or lost to follow-up, on any baseline measure of cognition or symptom severity. Participants who did not complete the trial were similar on every demographic variable to those who did, except for father’s ethnicity, with participants who did not complete the trial being more likely to have a father from a non-Anglo-Saxon background. Reasons for this are unclear, given that there was no difference on mothers’ ethnicity or any other parental demographic variable. Overall, this study had a large, reasonably representative sample of children with autism who completed the study.

### 5.3.3 Problems with adherence

An inclusion criterion of the study was the expected ability to take the trial medication (i.e. by swallowing capsules or having the contents mixed into a drink). While this was confirmed at baseline, anecdotally, many parents/guardians reported difficulty in administering the capsules to participating children. For some participants, several daily attempts may have been required before successful capsule delivery was achieved, sometimes including mixing capsule contents into different types of drinks or re-attempting delivery at different times of day. This could be a time-consuming and frustrating process, and doses may have been missed as a result. Any changes to the
NAC treatment following dissolution could not be confirmed. However, NAC is relatively stable and this should not have confounded the study.

It was intended that adherence to study protocol be measured by capsule return, but this proved complex due to the delivery issues detailed above. It is difficult to know how reliably participants were receiving their trial medication, and any effects this may have had on study outcomes. Possible solutions to this problem in future studies could include having parents/guardians keep a log of successful and unsuccessful administrations of trial medication, although this would increase burden of participation; or considering electronic pill boxes. More tolerable formulations could also be useful. Should NAC ultimately become a standard treatment in autism, investigation into the use of alternative delivery methods may be warranted, given the sensory and dietary issues frequently experienced in children with autism.

5.3.4 Outcome measures and analysis

This study used a range of comprehensive, valid measures to assess changes in autism symptomatology, including measures of all three of the core symptom domains. Due to the poorly understood aetiology of autism, its wide heterogeneity and uncertainty as to which symptoms if any NAC may affect, all core symptom domains, as well as associated adaptive and problem behaviours, were investigated. With a sample of 98 participants, the study was powered to detect a moderate-large effect size of $d=.57$. Given the null results of this trial, future studies may need to power their sample size against a smaller effect size.

Three out of four previous pilot trials of NAC in autism found that NAC improved scores on the Irritability subscale of the ABC, while the fourth found no changes on this subscale (7-10). The current study did not use the ABC, instead using the DBC-P, which similarly aims to assess problem behaviours and thus contains many parallel items (321). Analysis of DBC-P items corresponding as closely as possible to the Irritability subscale of the ABC did not yield any significant differences between NAC and placebo groups.
Aside from the Clinical Global Impressions scales, outcome measures relied solely on parent/guardian report of symptoms. Fatigue from completing multiple questionnaires could have affected the reliability of responses, although this may have been balanced by the use of qualitative information collected during the interview. Parental expectation bias and placebo effects may also have played a role in the results of this study, given that both the NAC and placebo groups demonstrated significant improvements over time on many outcome measures. As well as possible reporting bias, natural advances in development and regression to the mean were almost certain to occur in both groups over time. The placebo group in fact showed improvements on more measures than the NAC group, though there were no significant differences between groups on any measure.

5.3.5 *Lack of biological measures*

Another limitation was the absence of biological specimens in this study. The decision was made not to include such measures, due to concerns that a requirement for blood tests and other physiological tests may have been a deterrent to participation for some families, by increasing participant burden or making it more difficult for children with sensory issues to take part. Similarly, if this had been included as an option, given the sample size of the study, it was expected that any lack of participation would decrease the power of biological investigations. This would therefore be additional participant burden without sufficient scientific merit.

The 2016 NAC trial by Wink et al. (10) is the only one to date that has examined changes in physiological markers of glutathione functioning and oxidative stress in children with autism. It included measures of reduced and oxidised glutathione (GSH and GSSG), homocysteine and DNA damage, based on whole blood samples taken before and after treatment with 60 mg/kg/day of NAC or placebo. The study found that, as expected, NAC increased GSH levels, though there were no differences between NAC and placebo groups on any other oxidative biomarker, and no changes in behavioural symptoms (10).
In the current study, it is impossible to know whether the 500 mg daily dose of NAC had any effect on glutathione levels or any of its other neurobiological targets such as glutamate, inflammation, neurogenesis or mitochondrial function. If changes in glutathione or other targets were evident, the null results of this study could have been much more informative about the relationship, or lack thereof, between oxidative stress and autism symptomatology. It remains unknown whether this relatively low dose of NAC simply was insufficient to effect the physiological changes necessary for behavioural changes.

5.4 Implications and considerations for future research

This mixed-method, double-blind, randomised, placebo-controlled trial was the largest and longest of any trial of NAC in autism to date. The lack of significant findings on all quantitative measures suggests that this dose, 500 mg daily, is not effective in improving behavioural symptoms in children with autism. However, the qualitative findings in this study were encouraging, potentially indicating a signal towards improvements in calmness/irritability and verbal communication on post hoc analysis. It needs to be noted that such analyses are prone to type I error, but it is of interest that a positive finding with qualitative analyses in the current study aligns with the now extant literature. The clearly negative primary results may reach statistical significance with a higher dose of NAC, a more severely impaired and homogeneous sample, or a younger and larger sample.

This study highlights the potential utility and feasibility of mixed-methods clinical trials. Use of qualitative methods in this study required little extra resources except for transcription and analysis time, as the qualitative data was gathered during the course of normal study visits. Autism seems to be a particularly promising area for mixed-methods treatment research, given the current lack of proven, biologically based treatments with clear target symptoms. With such heterogeneity in clinical presentation, participants in clinical trials may respond to novel treatments in varying and unexpected ways that may not be detected using standardised outcome measures and traditional hypothesis testing (325).
It is difficult to comment on the implications of the overall study results for the role of oxidative stress in autism. As discussed above, the dosage may simply have been insufficient to effect physiological and behavioural changes. Four pilot studies, published after the commencement of the current study, suggest that NAC may be safe and tolerable at doses up to 2700 mg daily in children with autism (7-10). Its efficacy in improving irritability is also supported by three of those four trials (7-9), and still warrants further investigation in larger studies using similar doses. If replicated in larger RCTs, future studies may focus on dose finding and trials in adolescents and adults with autism. It is also uncertain if any effects on irritability are specific to autism or may be seen in other disorders as well. It may also be worth further exploring the qualitative findings of improvement to verbal communication in the current study, which had not been investigated in any prior RCT. It will also be important to use biomarkers to further elucidate the physiological, neurochemical and other mechanisms by which NAC may be effective.

Evidence continues to accumulate for the presence of abnormalities in redox biology in people with autism, though it is still uncertain whether oxidative stress is a cause of autism, a contributor to the illness or a consequence of the illness. There remains a need for prospective, population-based studies using comprehensive biomarker measurement before these questions can be answered. Despite the null quantitative results of the current study, interventions targeting oxidative pathways remain a promising area for future research.

5.5 Conclusion

Extant evidence suggests that oxidative stress and glutathione deficiency may have a role in the pathology of autism. N-acetyl cysteine (NAC) is a glutathione precursor, which pilot studies suggest may be effective in improving irritability in children with autism. However, in this randomised, placebo-controlled trial in 98 children with autism, 500 mg daily of NAC was safe and tolerable, but had no effect on primary or secondary outcomes, including communication, social interaction, repetitive behaviours and adaptive functioning. Qualitative analyses found that, compared to the placebo group, children receiving NAC showed significantly more improvements to calmness.
and verbal communication. This study does not support the widespread use of NAC for autism, although questions remain regarding dosage, and effects on specific symptoms within the broader clinical picture.
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Appendix A. Barwon Health HREC Approval Letter

HUMAN RESEARCH ETHICS COMMITTEE

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ETHICS COMMITTEE APPROVAL STATEMENT

Project Number 09/141
Site Barwon Health
Principal Investigator: Michael Berk
Title: EFFICACY OF N-ACETYL CYSTEINE IN AUTISM: A DOUBLE-BLIND, PLACEBO-CONTROLLED RANDOMISED TRIAL
Co investigators Olivia Dean, Seetal Dodd, Prof. Bruce Tonge, Killie Gray, Avril Brereton
Student names Kristi Villagonzola

Thankyou for submitting your application with the Human Research Ethics Committee.

Full approval was granted on 10/06/2010 for three years or until the anticipated completion date, 1/08/2012, whichever is the closer.

In addition any items approved in support of this project are listed below:

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The Barwon Health Human Research Ethics Committee (HREC) operates in accordance to guidelines established by the National Health and Medical Research Council, National Statement on Ethical Conduct in Human Research (2007).
I have attached a current list of the HREC membership. Committee members are required to
disclose any actual or potential conflict of interest in the research under consideration.
Members who do disclose a conflict, such as involvement in the research team, are not
permitted to participate in the deliberations or decisions relating to the approval of this project.

Please note your annual report is in the month of: Jun

It is now your responsibility to undertake the following:

1. To inform any personnel who should be aware of this project

2. To ensure, if applicable, that accurate documentation of the consent process is recorded
   in the participant’s hospital history and that a photostated copy of the consent form is also
   placed in the hospital history.

3. To advise the Committee, in writing, of any changes you wish to make to the running of the
   project, including extending beyond the anticipated completion date.

To extend approval please indicate on the report form and, if your study involves
participants, attach the current participant information and consent form.

4. To advise the Committee, in writing, of any serious adverse events

5. To supply written annual reports advising of the progress of the project and a final report
   advising of completion

6. To ensure that, if applicable, the project is registered on a Clinical Trials Registry and that
   the number is made available to the Committee for cut-off records

In all future correspondence regarding your study please quote your project number and full

title of your project.

Please note: Research projects to be undertaken at private institutions are not covered
by the Barwon Health Medical Malpractice Policy.

In the case of medical research, care should be taken to ensure that the investigator’s medical
insurance policy is current and the institute in which the research is conducted is adequately
insured.

It is the responsibility of the investigator to ensure adequate coverage in the event of
litigation

10/06/2010  Project Number  09/141  Page 2 of 3

The Barwon Health Human Research Ethics Committee (HREC) operates in accordance with guidelines established by the National
Should you require any further information concerning the Committee's approval of your research or have any concerns regarding the reporting requirements please contact the Research and Ethics Office on:

Phone  03 5226 7978
Fax  03 5260 3023
Address  Barwon Health, P.O. Box 281, Geelong, Victoria 3220
Email  hrec@barwonhealth.org.au
Website: www.barwonhealth.org.au

On behalf of the Committee, best wishes for your project.

Yours sincerely,

SIMON FRENCH
Chair
Human Research Ethics Committee
Appendix B. Participant Information and Consent Form

PARENT/GUARDIAN
Information and Consent Form

Study Title: Efficacy of N-Acetyl Cysteine in Autism: A double blind, placebo-controlled randomised trial.

Funding Body: Australian Rotary Health Fund

Investigators: Prof. Michael Berk (Principal Investigator), Dr. Seetal Dodd, Dr. Olivia Dean, Prof. Bruce Tonge (Monash University), Dr. Kylie Gray (Monash University), Dr. Avril Breton (Monash University), Ms. Kristi Villagonzalo

Introduction

You are invited to permit your child to participate in a study that is investigating the treatment effects of an antioxidant in Autism. Your child is eligible to participate in this study because they have an Autism Spectrum Disorder (ASD). Before you agree that your child can participate in this research study, it is important that you read and understand the following explanation of the study. It describes the purpose, procedures, benefits, risks, discomforts, and precautions associated with the study. It describes alternative treatments that are available to you and your child’s rights as a participant, including the right to withdraw from the study at any time. It is important to understand that no guarantees or assurances can be made regarding the results of the study. It is also important to understand that refusal to participate will not influence the usual treatment your child receives.

This consent may contain words that you do not understand. Please ask the research staff to explain any words or information that you do not understand. It is essential that you are completely truthful regarding the health history of your child and any symptoms or reactions they may experience during the study.

If you decide you would like your child to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read;
- Consent for your child to take part in the research project;
- Consent to your child receiving the treatment that is described.
Background
Your child has been invited to participate in a research study under the direction of Professor Michael Berk because they have been diagnosed with ASD. This study involves the use of a naturally occurring substance called N-acetyl Cysteine (NAC). NAC is an amino acid found in protein-containing foods. It is approved by the Australian Therapeutic Goods Administration (TGA) for the treatment of paracetamol overdose and as a mucolytic treatment for cystic fibrosis. However, it is not approved to treat ASD. Its use in this study is therefore experimental. This study plans to determine if it is an effective treatment for ASD.

This study is being supported by Australian Rotary Health Fund. The results of this trial will form the basis for Ms Kristi Villagonzalo’s PhD dissertation, and may also be published in the form of journal articles and conference presentations. A summary of results will be provided to you directly.

Purpose
N-acetyl Cysteine (NAC) is an amino acid found naturally in protein-containing food such as meat, fish and green leafy vegetables. It is also used in health foods and over-the-counter cough medications. NAC works as an antioxidant (a substance that prevents or slows the breakdown of other substances by oxygen). There is reason to believe that there are insufficient levels of antioxidants in children diagnosed with ASD. This could form part of the disease process of autism. NAC has other effects. It has anti-inflammatory effects and also promotes the growth of nerve cells. It is unclear if these properties will contribute to the therapeutic effects of NAC. The purpose of this trial is to research the benefits of adding NAC to the usual treatment in children who suffer from ASD. We believe that the addition of NAC may improve the symptoms experienced by your child. NAC has few side effects. If they do occur, they may include sedation, intestinal complaints and the aggravation of asthma.

Procedures
During the trial your child will either receive NAC capsules or dummy capsules called placebo capsules (containing corn starch). Your child will take these in addition any other usual medications or treatments your child may need. Being on this trial will not influence these other treatments. You will be asked to administer one capsule per day of either NAC or placebo to your child. If you child does not readily take capsules you can add the contents of the capsules (by carefully breaking open the capsules and emptying the contents) into a glass containing cold milk and a heaped teaspoon (20 g) of a chocolate flavoured mild drink (such as Milo), or orange juice. The duration of the treatment is six months. You will be asked to bring your child in for a baseline interview and then again at months one, three and six. In addition, a trial clinician will contact you by telephone on at least a monthly basis between
these visits to see how you and your child are going. Following this, you will be asked to bring your child in for two additional interviews, six months and one year following the end of the treatment phase. At each visit the trial will ask you and your child about how they are feeling and several questionnaires will be completed.

With your consent, researchers may access relevant information in your child’s medical records. This will include paediatricians’ reports about your child’s autism diagnosis and the assessments they used to reach this diagnosis. All data collected will be de-identified once entered into our database, and only overall group data will be reported.

Participation in the study is voluntary and you are free to refuse to consent for your child to participate. You may withdraw consent at any time. If you choose for your child not to participate, your child will not be disadvantaged in any way.

Precautions
You must tell your study clinician about any medical treatments that your child may have to receive or undergo during the study period (such as elective surgery).

Risks and Discomforts
The possible side effects seen with oral NAC consumption include aggravation of asthma, nausea, and other gastrointestinal symptoms (including diarrhoea or constipation) stomatitis (inflammation of the mouth), rhinorrhea (runny nose), fever, and sedation. These tend to be uncommon and when they do occur they are usually very mild. Previous trials by our team did not show any side effects being present more commonly in people using NAC than in people given placebo.

As this is an experimental use of the study drug, there may be additional unknown or unforeseen risks or effects on symptoms.

Significant New Findings
All new findings discovered during the course of this research that may reasonably influence your willingness to allow your child to continue to participate in this study will be made known to you as soon as they become available.

Possible Benefits
N-acetyl cysteine may be an effective treatment for autism spectrum disorders. Previous research by our unit has shown clear benefits of NAC treatment in people with schizophrenia and bipolar disorder. However, benefits cannot be promised, nor can the chance of benefit.
be accurately predicted. Furthermore, by participating in this study your child will be contributing to a scientific investigation that may assist other children in the future.

The costs for study medication and study procedures will be paid for by the Australian Rotary Health Fund.

Alternative Treatments
You do not have to allow your child to participate in this research study to receive treatment for their condition. Other treatment options are available. You should discuss these alternative treatments with your own doctor and research staff.

Voluntary Participation and Withdrawal
Your child’s participation in this research study is voluntary. You may choose not to allow your child to participate, or you may decide to withdraw your consent and discontinue their participation from this study at any time without penalty or loss of benefits. As participation is voluntary, you or your child will not be paid for your time. Your withdrawal will not jeopardize the medical care of your child at this facility. If you wish to withdraw your child from the study, please notify research staff immediately and arrange for a final study visit.

Your study clinician may end your child’s participation in this research program for any reason that they may feel is appropriate. These may include but are not limited to an adverse event, injury, a medical condition which may place your child at risk of further complications if they continue to participate, failure to take the medication as instructed, or termination of the study by the investigators or for other administrative reasons.

Injury During Trial
In the event that your child suffers an injury as a result of participating in this trial, it is important that you contact your study clinician as soon as possible. If necessary, hospital care and treatment will be provided at no extra cost if you elect for your child to be treated as a public patient at the public health service.

If you would like your child to continue with this medication at the end of the trial, it is available by purchase on the internet and selected pharmacies. Please ask your trial clinician for further details if you are interested in purchasing NAC.

Confidentiality and Access to Your Records
Records of your child’s participation in this study will be held confidential so far as permitted by law. This will include de-identification of all documents relating to the trial by assigning
your child a random number that will identify them in terms of a data set and not an individual. However, your study clinician, research investigators, regulatory agencies, and the Barwon Health Research Ethics Committee, will be able to inspect and have access to confidential data that identifies your child by name. Any analysis, interpretation and publication of the study results will not identify your child. You have the right to access, and to correction of, information collected during this trial pursuant with the Freedom of Information Act 1982 (Vic). By consenting to your child’s participation in this trial, you also consent to the release of your medical records to the above named persons/bodies. Records relating to the results of the study will be kept for 23 years, and you may access that information if required.
Questions
The outline of the research study has been described to you in this consent form. For additional information and answers to questions regarding this research study or if your child experience any medical problems, please contact:

Professor Michael Berk: 03 4215 3330
Dr. Seetal Dodd: 03 4215 3299
Ms. Kristi Villagonzalo: 03 4215 3301

If you seek emergency care for your child, or if hospitalisation is required, please alert the treating physician that you are enrolled in a research study being conducted by Professor Berk.

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about your rights as a research participant, then you may contact:

Bernice Davies, Secretary, Human Research Ethics Committee
Ph: (03) 4215 3372

You will need to tell Bernice the name of one of the researchers given in the section above.
**PARENT/GUARDIAN CONSENT FORM**

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I have been asked that my son/daughter/person for whom I am the guardian to participate in the research project entitled “Efficacy of N-Acetyl cysteine in autism: A double-blind, placebo controlled randomised trial” being conducted by Prof. Michael Berk, Dr. Seetal Dodd, Dr. Olivia Dean, Prof. Bruce Tonge, Dr. Kylie Gray, Dr. Avril Breerton and Ms. Kristi Villagonzalo.

**Name of child**

I give voluntary consent for my son/daughter/person for whom I am the guardian to participate in the above project. I have had the project explained to me, and I have read the Explanatory Statement, which I keep for my records. I understand that the research study will be carried out in a manner conforming to the principles set out by the National Statement on Ethical Conduct in Research Involving Humans, and further that:

1. I understand the general purposes, methods, demands and benefits and possible risks, inconveniences and discomforts of the study as outlined in the 'Parent/Guardian Information Sheet' that has been given to me or my son/daughter/person for whom I am the guardian.

2. Although I understand that the purpose of this research project is to improve the treatment of ASD, it has been explained that my involvement may not be of any direct personal benefit to me or my son/daughter/person for whom I am the guardian.

3. My participation in the research study is voluntary, and I am free to withdraw at any time, and to continue receiving appropriate treatment for my son/daughter/person for whom I am the guardian, as will be the case if I do not volunteer to enter the study.

4. I have been given the opportunity to have a member of my family or a friend present while the project was explained.

5. I have been given the opportunity to ask questions in relation to the research study, and I have received all the information and explanations I have requested.

6. I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party.

**Parent/Guardian Signature**

**Date**

**Witness - Name (print) and Signature**

**Date**

**Investigator - Name (print) and Signature**

**Date**

Participant Information and Consent Form
Version #6 Dated 17th March 2011
Barwon Health

Page 7 of 7
Appendix C. Interview Forms and Outcome Measures

N-ACETYL CYSTEINE IN AUTISM TRIAL

Site Number __ Interviewer Initials __ __
Subject Initials __ __ __ Subject Number __ __ __
Date __ __ __ VISIT 1 - BASELINE

PARTICIPANT DETAILS

Child’s name: ________________________________

Carer’s name: ______________________________
Relationship to child: ______________________

Are you this child’s primary carer?
☐ Yes
☐ No  Who is the child’s primary carer? ______________________________

Mother’s name: ______________________________
Contact no.: H: __________ W: __________ M: __________

Father’s name: ______________________________
Contact no.: H: __________ W: __________ M: __________

Alternative contact (e.g. grandparent or other relative)
Name: ______________________________
Relationship to child: ______________________
Contact no.: H: __________ W: __________ M: __________
N-ACETYL CYSTEINE IN AUTISM TRIAL

Site Number __ interviewee initials __
Subject initials __ __ __
Date __ __ __ __

VISIT 1 - BASELINE

Is this child currently attending a primary school (mainstream or special)?
☐ No
☐ Special school
☐ Mainstream school

Does the child have a classroom aide? ☐ No
☐ Yes

Hours/day ________
Days/week ________

If this child is not attending school, does this child regularly attend:
☐ Child-care centre Days/week ___________ Hours/day ________
☐ Playgroup Days/week ___________ Hours/day ________
☐ Kindergarten Days/week ___________ Hours/day ________
☐ Early intervention programme Days/week ___________ Hours/day ________

Name of school/child-care centre/kindergarten: ________________________________
Contact person e.g. teacher: ________________________________
Phone/fax number: ________________________________
Address: ________________________________

Name of school/child-care centre/kindergarten: ________________________________
Contact person e.g. teacher: ________________________________
Phone/fax number: ________________________________
Address: ________________________________

NAC IN AUTISM - DEMOGRAPHICS 2 of 15
N-ACETYL CYSTEINE IN AUTISM TRIAL

Site Number __________

Interviewer Initials __________

Subject Initials __________

Subject Number __________

Date __________

VISIT 1 - BASELINE

DEMOGRAPHICS – CHILD

Date of Birth: ___/___/_______ (DD/MM/YYYY)

Country of birth: ______________________________________

Gender: □ Male □ Female

Height: ___________ cm OR __________ ft. __________ in.

Weight: ___________ kg OR ___________ lb

History

What was this child’s weight at birth? ___________ kg OR ___________ lb

Were there any complications during the child’s birth?

□ No

□ Yes (please specify) ______________________________________

________________________________________________________________________

At what stage of the pregnancy was this child born? ___________ weeks

Were there any problems with the pregnancy? (Including any illness experienced by the child’s mother during the pregnancy)

□ No

□ Yes (please specify) ______________________________________

________________________________________________________________________
N-ACETYLCysteine in Autism trial

Site Number ___  Interviewer Initials ___
Subject Initials ___ ___  Subject Number ___ ___
Date ___ ___  VISIT 1 - BASELINE

Age when diagnosed with autism: _____ yrs _____ months

When did you first become concerned about your child’s behaviour or development?

How old were they then? _____ yrs _____ months

What were you concerned about at that time? (Tick all that apply.)

☐ Communication deficits  Please specify:

☐ Social interaction  Please specify:

☐ Repetitive behaviours  Please specify:

☐ Motor difficulties or delays  Please specify:

☐ General developmental difficulties  Please specify:

☐ Other  Please specify:

Other medical conditions: ____________________________________

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Loss of language skills

Has your child ever lost previously acquired language skills?

☐ No (proceed to next section on Loss of other skills)
☐ Yes  Age at loss: _____ yrs _____ months

How much language did your child have before losing it? (Specify meaningful words, extent of spontaneous usage and level of communicative use.) ________________________________

___________________________________________________________________________

___________________________________________________________________________

Clinician to determine previous level of language skills:

☐ Daily, spontaneous and meaningful speech – at least five words used regularly for at least three continuous months
☐ Occasional and/or fewer than five words used spontaneously and communicatively
☐ Speech or sound upon request (with or without spontaneous imitation)
☐ Spontaneous imitations of vocalisation without elicited imitation or spontaneous communicative speech

What aspects of language did your child lose?

☐ Spontaneous use of at least five meaningful words  Comments: _______________________

___________________________________________________________________________

☐ Use of language to communicate with others  Comments: _______________________

___________________________________________________________________________

☐ Syntactical skills (grammar)  Comments: _______________________

___________________________________________________________________________

☐ Articulation (pronunciation)  Comments: _______________________

___________________________________________________________________________
Has your child regained their language skills?
☐ Yes, to same level as before or higher  Age at recovery: _____ yrs _____ months
☐ Yes, but not to the same level as before  Age at recovery: _____ yrs _____ months
☐ Loss still present without recovery of language functions
☐ Progressive deterioration continuing

Loss of other skills
Has your child ever lost any other previously acquired skills?
☐ No (proceed to next section on Other Interventions)
☐ Gross motor skills (e.g. posture, gait, coordination)  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________

☐ Fine motor skills (e.g. ability to grip/hold objects)  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________

☐ Self-help skills (e.g. feeding, dressing, bathroom)  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________

☐ Constructive or imaginative play  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________

☐ Social engagement and responsiveness  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________

☐ Other  Age at loss: _____ yrs _____ months
Comments: ___________________________________________________________________________
Other Interventions

Is this child currently receiving or has the child ever received any other forms of intervention for autism? If so, at what age did they commence/cease this intervention? How much time do/did they spend undertaking this intervention?

☐ None

☐ Speech therapy
☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Hours/week _________ Days/week __________

☐ ABA programme
☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Hours/week _________ Days/week __________

☐ Early Intervention Programme
☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Hours/week _________ Days/week __________

☐ Special school
☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Hours/week _________ Days/week __________

☐ Occupational therapy
☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Hours/week _________ Days/week __________
N-ACETYL CYSTEINE IN AUTISM TRIAL

Site Number ____  Interviewer Initials ____
Subject Initials ____  Subject Number ____
Date ____  VISIT 1 - BASELINE

☐ Physiotherapy  ☐ Current  ☐ Past
  Age commenced ____ yrs ____ months  Ceased ____ yrs ____ months
  Hours/week _________ Days/week _________

☐ Other (please specify)
  • __________________________  ☐ Current  ☐ Past
    Age commenced ____ yrs ____ months  Ceased ____ yrs ____ months
    Hours/week _________ Days/week _________
  • __________________________  ☐ Current  ☐ Past
    Age commenced ____ yrs ____ months  Ceased ____ yrs ____ months
    Hours/week _________ Days/week _________
Is this child currently receiving or has the child ever received any complementary/alternative therapies? These may include nutritional supplements, special diets (e.g. gluten or casein free), massage or touch based therapy, chiropractic therapy, osteopathy, etc. At what age did they commence/cease undertaking this therapy?

☐ None

Name ________________________________  ☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Comments: ________________________________________________________________

Name ________________________________  ☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Comments: ________________________________________________________________

Name ________________________________  ☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Comments: ________________________________________________________________

Name ________________________________  ☐ Current  ☐ Past
Age commenced _____ yrs _____ months  Ceased _____ yrs _____ months
Comments: ________________________________________________________________
**Is this child currently taking or has the child ever taken any medications?** If so, what is/was their dose? (Please note below if the medication is taken on an as-needs basis only, e.g. asthma medication.) At what age did they commence/cease taking this medication?

- None

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<th>Name</th>
<th>Current</th>
<th>Past</th>
<th>Age commenced</th>
<th>Ceased</th>
<th>Dose mg/day</th>
<th>Taken on as-needs basis only</th>
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- Name
- Age commenced ___ yrs ___ months
- Ceased ___ yrs ___ months
- Dose ________ mg/day
- Taken on as-needs basis only

- Name
- Age commenced ___ yrs ___ months
- Ceased ___ yrs ___ months
- Dose ________ mg/day
- Taken on as-needs basis only

- Name
- Age commenced ___ yrs ___ months
- Ceased ___ yrs ___ months
- Dose ________ mg/day
- Taken on as-needs basis only

- Name
- Age commenced ___ yrs ___ months
- Ceased ___ yrs ___ months
- Dose ________ mg/day
- Taken on as-needs basis only

- Name
- Age commenced ___ yrs ___ months
- Ceased ___ yrs ___ months
- Dose ________ mg/day
- Taken on as-needs basis only
Family

Does this child have any biological siblings?

Number: _______ Brother(s) _______ Sister(s)
Ages: __________________________

Does this child have any step-siblings?

Number: _______ Brother(s) _______ Sister(s)
Ages: __________________________

Does this child have any adoptive siblings?

Number: _______ Brother(s) _______ Sister(s)
Ages: __________________________

Do any of the child’s siblings have autism or another developmental disorder (e.g. Asperger’s syndrome; intellectual delay, etc.)?

□ No

□ Yes (please specify number of siblings with diagnosis and their diagnosis) __________________________
________________________________________________________
________________________________________________________
________________________________________________________

Do any of the child’s other relatives have autism or another developmental disorder (e.g. Asperger’s syndrome; intellectual delay, etc.)?

□ No

□ Yes (please specify number of relatives with diagnosis, their diagnosis and their relationship to this child) __________________________
________________________________________________________
________________________________________________________
________________________________________________________
________________________________________________________

NAC IN AUTISM - DEMOGRAPHICS
Who does this child live with most of the time? (Tick all that apply.)

- Biological mother
- Biological father
- Step-parent(s)
- Adoptive/foster parent(s)
- Grandparent(s)
- Sibling(s)
- Other relative(s) e.g. uncle, aunt, cousins
- Other (please specify) ____________________________________________________________

Are any languages other than English spoken to the child?

- No
- Yes (please specify) ____________________________________________________________
DEMOGRAPHICS – CARER(S)

Please answer each of these questions about this child’s biological mother and father to the best of your ability. If you are the child’s primary carer, but are not the child’s mother or father, please also answer these questions about yourself below.

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Father</th>
<th>Yourself</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth:</td>
<td></td>
<td></td>
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<tr>
<td>(DD/MM/YYYY)</td>
<td><em><strong><strong><strong>/_____/</strong></strong></strong></em></td>
<td><strong><strong>/_____/</strong></strong>___</td>
<td><em><strong>/</strong></em>/_________</td>
</tr>
<tr>
<td>Was this person born in Australia?</td>
<td>☐ Yes</td>
<td>☐ Yes</td>
<td>☐ Yes</td>
</tr>
<tr>
<td></td>
<td>☐ No</td>
<td>☐ No</td>
<td>☐ No</td>
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<tr>
<td>Where were they born?</td>
<td></td>
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<td></td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
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<tr>
<td>How long have they lived in Australia?</td>
<td>_____ yrs</td>
<td>_____ yrs</td>
<td>_____ yrs</td>
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<td></td>
<td>_____ months</td>
<td>_____ months</td>
<td>_____ months</td>
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<tr>
<td>Ethnic background:</td>
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<tr>
<td>(Tick all that apply)</td>
<td>☐ European</td>
<td>☐ European</td>
<td>☐ European</td>
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<tr>
<td></td>
<td>☐ Aboriginal/Torres Strait Islander</td>
<td>☐ Aboriginal/Torres Strait Islander</td>
<td>☐ Aboriginal/Torres Strait Islander</td>
</tr>
<tr>
<td></td>
<td>☐ Asian/Pacific Islander</td>
<td>☐ Asian/Pacific Islander</td>
<td>☐ Asian/Pacific Islander</td>
</tr>
<tr>
<td></td>
<td>☐ African/Middle Eastern</td>
<td>☐ African/Middle Eastern</td>
<td>☐ African/Middle Eastern</td>
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<tr>
<td></td>
<td>☐ Other (please specify) _______</td>
<td>☐ Other (please specify) _______</td>
<td>☐ Other (please specify) _______</td>
</tr>
<tr>
<td>Highest level of education completed:</td>
<td>☐ Year 8 or below</td>
<td>☐ Year 8 or below</td>
<td>☐ Year 8 or below</td>
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<tr>
<td></td>
<td>☐ Year 9/10/11</td>
<td>☐ Year 9/10/11</td>
<td>☐ Year 9/10/11</td>
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<tr>
<td></td>
<td>☐ Year 12</td>
<td>☐ Year 12</td>
<td>☐ Year 12</td>
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<tr>
<td></td>
<td>☐ TAFE certificate</td>
<td>☐ TAFE certificate</td>
<td>☐ TAFE certificate</td>
</tr>
<tr>
<td></td>
<td>☐ Diploma</td>
<td>☐ Diploma</td>
<td>☐ Diploma</td>
</tr>
</tbody>
</table>
**N-ACETYL CYSTEINE IN AUTISM TRIAL**

Site Number __  
Interviewer Initials __ __  
Subject Initials __ __ __  
Subject Number __ __ __  
Date __ ___ ____  

**VISIT 1 - BASELINE**

<table>
<thead>
<tr>
<th>Education Level</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>□ Bachelor’s degree (includes honours)</td>
<td></td>
<td>□ Bachelor’s degree (includes honours)</td>
<td></td>
</tr>
<tr>
<td>□ Postgraduate qualification</td>
<td></td>
<td>□ Postgraduate qualification</td>
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<tr>
<td>□ Other (please specify) __________</td>
<td></td>
<td>□ Other (please specify) __________</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Employment Status: (Tick all that apply)</th>
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<tbody>
<tr>
<td>□ Full time</td>
<td>□ Full time</td>
<td>□ Full time</td>
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<td>□ Part time</td>
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<td>□ Part time</td>
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<tr>
<td>□ Casual</td>
<td>□ Casual</td>
<td>□ Casual</td>
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<tr>
<td>□ Self-employed</td>
<td>□ Self-employed</td>
<td>□ Self-employed</td>
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<tr>
<td>□ Homemaker</td>
<td>□ Homemaker</td>
<td>□ Homemaker</td>
<td></td>
</tr>
<tr>
<td>□ Student</td>
<td>□ Student</td>
<td>□ Student</td>
<td></td>
</tr>
<tr>
<td>□ Looking for work</td>
<td>□ Looking for work</td>
<td>□ Looking for work</td>
<td></td>
</tr>
<tr>
<td>□ Unemployed; not looking for work</td>
<td>□ Unemployed; not looking for work</td>
<td>□ Unemployed; not looking for work</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current/usual occupation:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
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</table>

**What is the relationship between this child’s biological mother and father?**

□ Married  
□ De-facto  
□ In a relationship but living separately  
□ Separated  
□ Divorced  
□ Other (please specify) ________________________________
What is the total (after tax) yearly household income in the household where the child lives?

☐ $0-20,000
☐ $20,001-40,000
☐ $40,001-60,000
☐ $60,001-80,000
☐ $80,001-100,000
☐ $100,001-120,000
☐ $120,001 or more
☐ Would rather not say
☐ Don’t know
N-ACETYLCYSTEINE IN AUTISM TRIAL

Site Number ___                               Interviewer Initials ___
Subject Initials ___ ___                       Subject Number ___ ___
Date ___ ___                                  VISIT X – WEEK X

UPDATE SINCE LAST VISIT

Height: ___________ cm  OR  ______ ft. _______ in.
Weight: ___________ kg  OR  ___________ lb

Have any of your child’s circumstances changed significantly in the past X weeks (excluding changes in medication or autism interventions)?

Change in school situation
☐ No  ☐ Yes (please specify) __________________________

Change in family situation
☐ No  ☐ Yes (please specify) __________________________

Change in living situation
☐ No  ☐ Yes (please specify) __________________________

Other important change
☐ No  ☐ Yes (please specify) __________________________
In the past X weeks, has your child:

**Commenced any new medications?**  □ No  □ Yes (please specify below)

**Stopped any medications?**  □ No  □ Yes (please specify below)

**Changed the dose of any medications he/she was already taking?**  □ No  □ Yes (please specify below)

<table>
<thead>
<tr>
<th>Name</th>
<th>Commenced</th>
<th>Stopped</th>
<th>Changed dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Dose</td>
<td>□ mg/day</td>
<td>□</td>
<td>□</td>
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<tr>
<td>Reason for change</td>
<td></td>
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<th>Changed dose</th>
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<td>Dose</td>
<td>□ mg/day</td>
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<td>Reason for change</td>
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<tbody>
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<tr>
<td>Dose</td>
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<td>Reason for change</td>
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<tr>
<th>Name</th>
<th>Commenced</th>
<th>Stopped</th>
<th>Changed dose</th>
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<td>□</td>
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<td>□</td>
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<tr>
<td>Dose</td>
<td>□ mg/day</td>
<td>□</td>
<td>□</td>
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<tr>
<td>Reason for change</td>
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</tbody>
</table>
In the past X weeks, has your child:

Commenced any new autism interventions?  □ No  □ Yes (please specify below)
Stopped any autism interventions?  □ No  □ Yes (please specify below)
Changed the frequency of any autism interventions he/she was already involved in?
□ No  □ Yes (please specify below)

Type of intervention __________________________________________

□ Commenced  □ Stopped  □ Changed frequency
Reason for change __________________________________________

Type of intervention __________________________________________

□ Commenced  □ Stopped  □ Changed frequency
Reason for change __________________________________________

Type of intervention __________________________________________

□ Commenced  □ Stopped  □ Changed frequency
Reason for change __________________________________________

Type of intervention __________________________________________

□ Commenced  □ Stopped  □ Changed frequency
Reason for change __________________________________________

Type of intervention __________________________________________

□ Commenced  □ Stopped  □ Changed frequency
Reason for change __________________________________________
Has your child experienced any significant medical problems in the past X weeks? (This includes any illness that lasted two or more days, or required a visit to a doctor.)

☐ No
☐ Yes (please specify) ________________________________________________

__________________________________________________________

__________________________________________________________

__________________________________________________________

__________________________________________________________

Based on your child’s behaviour in the past X weeks, do you believe that he/she is taking the placebo or NAC treatment?

☐ Placebo
☐ NAC
☐ Don’t know

Why? _______________________________________________________

__________________________________________________________

__________________________________________________________

__________________________________________________________
N-ACETYLCYSTEINE IN AUTISM TRIAL

Site Number ___
Subject Initials ___ ___
Date ___

Interviewer Initials ___
Subject Number ___ ___
VISIT X – WEEK X

To be completed by the parent/carer.

PGI – IMPROVEMENT and/or Change from BASELINE

Compared to BASELINE, how much has your child changed?

☐ Very much improved
☐ Much improved
☐ Minimally improved
☐ No change
☐ Minimally worse
☐ Much worse
☐ Very much worse
N-ACETYL CYSTEINE IN AUTISM TRIAL

Site Number ___  Interviewer Initials ___
Subject Initials ___ ___  Subject Number ___ ___
Date ___ ___  VISIT X – WEEK X

To be completed by the clinician at the end of each visit.

**CGI – SEVERITY of symptoms**

Considering your total clinical experience with children with autism, how severely ill has the participant been in the past X weeks?

- Normal
- Minimally ill
- Mildly ill
- Moderately ill
- Markedly ill
- Severely ill
- Very severely ill

**CGI – IMPROVEMENT and/or Change from BASELINE**

Compared to BASELINE, how much has the participant changed?

- Very much improved
- Much improved
- Minimally improved
- No change
- Minimally worse
- Much worse
- Very much worse
A SERIOUS ADVERSE EVENT is defined as ANY untoward medical occurrence, of ANY degree of intensity, at ANY dose that: 1) results in death; 2) is life threatening; 3) requires hospitalization or prolongs an existing hospitalization; OR 4) results in persistent or significant disability or incapacity.

Please report any SERIOUS adverse event to the trial investigator IMMEDIATELY.
### Social Responsiveness Scale AutoScore™ Form

**Directions**
For each question, circle the number that best describes the child's behavior over the past 6 months.

**Child's Name:**

**Chronological Age:**

**Gender (required):**
- [ ] Female
- [ ] Male

**Ethnicity:**

**Respondent's Name:**

**Administration Date:**

**Relationship to Child:**
- [ ] Mother
- [ ] Father
- [ ] Other

---

**PLEASE PRESS HARD WHEN MARKING YOUR RESPONSES.**

<table>
<thead>
<tr>
<th>Item</th>
<th>1 = NOT TRUE</th>
<th>2 = SOMETIMES TRUE</th>
<th>3 = OFTEN TRUE</th>
<th>4 = ALMOST ALWAYS TRUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>31.</td>
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<td>32.</td>
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</table>

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*Continue on back page.*

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183
<table>
<thead>
<tr>
<th></th>
<th>NOT TRUE</th>
<th>SOMETIMES TRUE</th>
<th>OFTEN TRUE</th>
<th>ALMOST ALWAYS TRUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.</td>
<td>Is socially awkward, even when he or she is trying to be polite.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>34.</td>
<td>Avoids people who want to be emotionally close to him or her.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>35.</td>
<td>Has trouble keeping up with the flow of a normal conversation.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>36.</td>
<td>Has difficulty relating to adults.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>37.</td>
<td>Has difficulty relating to peers.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>38.</td>
<td>Responds appropriately to mood changes in others (e.g., when a friend’s or playmate’s mood changes from happy to sad).</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>39.</td>
<td>Has an unusually narrow range of interests.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>40.</td>
<td>Is imaginative, good at pretending (without losing touch with reality).</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>41.</td>
<td>Wanders aimlessly from one activity to another.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>42.</td>
<td>Seems overly sensitive to sounds, textures, or smells.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>43.</td>
<td>Separates easily from caregivers.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>44.</td>
<td>Doesn’t understand how events relate to one another (cause and effect) the way other children his or her age do.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>45.</td>
<td>Focuses his or her attention to where others are looking or listening.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>46.</td>
<td>Has overly serious facial expressions.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>47.</td>
<td>Is too silly or laughs inappropriately.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>48.</td>
<td>Has a sense of humor, understands jokes.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>49.</td>
<td>Does extremely well at a few tasks, but does not do as well at most other tasks.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>50.</td>
<td>Has repetitive, odd behaviors such as hand flapping or rocking.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>51.</td>
<td>Has difficulty answering questions directly and ends up talking around the subject.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>52.</td>
<td>Knows when he or she is talking too loud or making too much noise.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>53.</td>
<td>Talks to people with an unusual tone of voice (e.g., talks like a robot or like he or she is giving a lecture).</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>54.</td>
<td>Seems to react to people as if they are objects.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>55.</td>
<td>Knows when he or she is too close to someone or is invading someone’s space.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>56.</td>
<td>Walks in between two people who are talking.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>57.</td>
<td>Gets teased a lot.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>58.</td>
<td>Concentrates too much on parts of things rather than seeing the whole picture.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>For example, if asked to describe what happened in a story, he or she may talk only about the kind of clothes the characters were wearing.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>59.</td>
<td>Is overly suspicious.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>60.</td>
<td>Is emotionally distant, doesn’t show his or her feelings.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>61.</td>
<td>Is inflexible, has a hard time changing his or her mind.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>62.</td>
<td>Gives unusual or illogical reasons for doing things.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>63.</td>
<td>Touches others in an unusual way (e.g., he or she may touch someone just to make contact and then walk away without saying anything).</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>64.</td>
<td>Is too tense in social settings.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>65.</td>
<td>Stares or gazes off into space.</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Children’s Communication Checklist (CCC-2)

by D.V.M. Bishop

Please enter a number in the □ bar in the right hand column, as follows:
- 0 = less than once a week (or never)
- 1 = at least once a week, but not every day
- 2 = once or twice a day
- 3 = several times (more than twice) a day (or always)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gets mixed up between he and she so might say “he” when talking about a girl, or “she” when talking about a boy</td>
</tr>
<tr>
<td>2</td>
<td>Simplifies words by leaving out some sounds, e.g., “crocodile” pronounced as “cockdile”, or “stranger” as “staynger”</td>
</tr>
<tr>
<td>3</td>
<td>Appears anxious in the company of other children</td>
</tr>
<tr>
<td>4</td>
<td>Makes false starts, and appears to grope for the right words; e.g., might say “can I - can I - can - can I have an - have an ice-cream”</td>
</tr>
<tr>
<td>5</td>
<td>Talks repetitively about things that no-one is interested in</td>
</tr>
<tr>
<td>6</td>
<td>Forgets words s/he knows – e.g. instead of “rhinoceros” may say “you know, the animal with the horn on its nose…”</td>
</tr>
<tr>
<td>7</td>
<td>With familiar adults, seems inattentive, distant or preoccupied</td>
</tr>
<tr>
<td>8</td>
<td>Looks blank in a situation where most children would show a clear facial expression – e.g. when angry, fearful or happy</td>
</tr>
<tr>
<td>9</td>
<td>When given the opportunity to do what s/he likes, chooses the same favourite activity (e.g. playing a specific computer game)</td>
</tr>
<tr>
<td>10</td>
<td>Uses terms like “he” or “it” without making it clear what s/he is talking about. For instance, when talking about a film, might say “he was really great” without explaining who “he” is</td>
</tr>
<tr>
<td>11</td>
<td>Says things that s/he does not seem to fully understand (may appear to be repeating something s/he’s heard an adult say). So, for instance, a 5-year-old may be heard to say of a teacher “she’s got a very good reputation”</td>
</tr>
<tr>
<td>12</td>
<td>Mixes up words of similar meaning, e.g., might say “dog” for “fox”, or “screwdriver” for “hammer”</td>
</tr>
<tr>
<td>13</td>
<td>Is babied, teased, or bullied by other children</td>
</tr>
<tr>
<td>14</td>
<td>Does not look at the person s/he is talking to</td>
</tr>
<tr>
<td>15</td>
<td>Misses the point of jokes and puns (though may be amused by nonverbal humour such as slapstick)</td>
</tr>
<tr>
<td>16</td>
<td>Is left out of joint activities by other children</td>
</tr>
<tr>
<td>17</td>
<td>Gets mixed up between he/him or she/her, so might say “him is working” rather than “he is working”, or “her have a cake” rather than “she has a cake”</td>
</tr>
<tr>
<td>18</td>
<td>Uses favourite phrases, sentences or longer sequences in rather inappropriate contexts. E.g., might say “all of a sudden” rather than “then”, as in “we went to the park and all of a sudden we had a picnic”. Or might habitually start utterances with “by the way”</td>
</tr>
<tr>
<td>19</td>
<td>Gets confused when a word is used with a different meaning from usual; e.g. might fail to understand if an unfamiliar person was described as “cold” (and would assume they were shivering!)</td>
</tr>
<tr>
<td>20</td>
<td>Stands too close to other people when talking to them</td>
</tr>
<tr>
<td>21</td>
<td>Talks to people too readily; e.g. without any encouragement, starts up a conversation with a stranger</td>
</tr>
<tr>
<td>22</td>
<td>Talks about lists of things s/he has memorised e.g., the names of the capitals of the world, or the names of varieties of dinosaurs</td>
</tr>
<tr>
<td>23</td>
<td>Pronounces words in an over-precise manner: accent may sound affected or “put-on”, as if child is mimicking a TV personality rather than talking like those around him/her</td>
</tr>
<tr>
<td>24</td>
<td>Pronounces words in a babyish way, such as “chimbley” for “chimney” or “bokkle” for “bottle”</td>
</tr>
<tr>
<td>25</td>
<td>Can be hard to tell if s/he is talking about something real or make-believe</td>
</tr>
<tr>
<td>26</td>
<td>Moves the conversation to a favourite topic, even if others don’t seem interested in it</td>
</tr>
<tr>
<td></td>
<td>Description</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>27</td>
<td>Produces utterances that sound babyish because they are just 2 or 3 words long, such as “me got ball” instead of “I’ve got a ball” or “give dolly” instead of “give me the dolly”</td>
</tr>
<tr>
<td>28</td>
<td>Ability to communicate varies from situation to situation – e.g. may cope well when talking one-to-one with a familiar adult, but have difficulty expressing him/herself in a group of children</td>
</tr>
<tr>
<td>29</td>
<td>Leaves off beginnings or ends of words, e.g. says “roe” instead of “road” or “nana” instead of “banana”</td>
</tr>
<tr>
<td>30</td>
<td>Repeats back what others have just said. For instance, if you ask, “what did you eat?” might say, “what did I eat?”</td>
</tr>
<tr>
<td>31</td>
<td>Ignores conversational overtures from others (e.g. if asked, “what are you making?” does not look up and just continues working)</td>
</tr>
<tr>
<td>32</td>
<td>Mixes up words that sound similar, e.g. might say “telephone” for “television” or “magician” for “musician”</td>
</tr>
<tr>
<td>33</td>
<td>Hurts or upsets other children without meaning to</td>
</tr>
<tr>
<td>34</td>
<td>Takes in just 1-2 words in a sentence, and so misinterprets what has been said. E.g. if someone says “I want to go skating next week”, s/he may think they’ve been skating, or want to go now</td>
</tr>
<tr>
<td>35</td>
<td>It’s difficult to stop him/her from talking</td>
</tr>
<tr>
<td>36</td>
<td>Leaves off past tense – ed endings on words, so might say “John kick the ball” instead of “John kicked the ball”, or “Sally play over there” instead of “Sally played over there”</td>
</tr>
<tr>
<td>37</td>
<td>Tells people things they know already</td>
</tr>
<tr>
<td>38</td>
<td>Makes mistakes in pronouncing long words; e.g. says “vegetable” rather than “vegetable” or “television” rather than “telescope”</td>
</tr>
<tr>
<td>39</td>
<td>Fails to recognise when other people are upset or angry</td>
</tr>
<tr>
<td>40</td>
<td>Gets the sequence of events muddled up when trying to tell a story or describe a recent event. E.g. if describing a film, might talk about the end before the beginning</td>
</tr>
<tr>
<td>41</td>
<td>Is over-literal, sometimes with (unintentionally) humorous results. E.g., a child who was asked “Do you find it hard to get up in the morning” replied “No. You just put one leg out of the bed and then the other and stand up.” Another child who was told “watch your hands” when using scissors, proceeded to stare at his fingers.</td>
</tr>
<tr>
<td>42</td>
<td>Includes over-precise information (e.g. exact date or time) in his/her talk, e.g. when asked “when did you go on holiday” may say “13th July 1995” rather than “in the summer”</td>
</tr>
<tr>
<td>43</td>
<td>Leaves out “is”, and so says “Daddy going to work” rather than “Daddy’s going to work” or “Daddy is going to work”. Or might say “The boy big” rather than “The boy is big”</td>
</tr>
<tr>
<td>44</td>
<td>Mispronounces “th” for “s” or “w” for “r”. E.g. says “there” instead of “there” or “wabbit” instead of “rabbit”</td>
</tr>
<tr>
<td>45</td>
<td>Asks a question, even though s/he has been given the answer</td>
</tr>
<tr>
<td>46</td>
<td>Is vague in choice of words, making it unclear what s/he is talking about, e.g. saying “that thing” rather than “kettle”</td>
</tr>
<tr>
<td>47</td>
<td>Shows interest in things or activities that most people would find unusual, such as traffic lights, washing machines, lamp-posts</td>
</tr>
<tr>
<td>48</td>
<td>Doesn’t explain what s/he is talking about to someone who doesn’t share his/her experiences; for instance, might talk about “Johnny” without explaining who he is</td>
</tr>
<tr>
<td>49</td>
<td>Surprises people by his/her knowledge of unusual words – uses terms you’d expect to hear from an adult rather than child</td>
</tr>
<tr>
<td>50</td>
<td>It is hard to make sense of what s/he is saying (even though the words are clearly spoken)</td>
</tr>
</tbody>
</table>
Children's Communication Checklist (CCC-2) by D.V.M. Bishop

Please enter a number in the box in the right hand column, as follows:
0 = less than once a week (or never); 1 = at least once a week, but not every day
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The questions so far have asked about difficulties children may have that affect communication. The remaining questions ask about communicative strengths.

Please respond 0 to 3, as before, but remember that now a 0 response would mean that a child lacks this strength, and a 3 would indicate good communicative skill.

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>Speaks clearly so that the words can easily be understood by someone who doesn't know him/her very well</td>
</tr>
<tr>
<td>52</td>
<td>Reacts positively when a new and unfamiliar activity is suggested</td>
</tr>
<tr>
<td>53</td>
<td>Talks clearly about what s/he plans to do in the future (e.g. what s/he will do tomorrow, or plans for going on holiday)</td>
</tr>
<tr>
<td>54</td>
<td>Appreciates the humour expressed by irony. Would be amused rather than confused if someone said &quot;Isn't it a lovely day?&quot; when it is pouring with rain</td>
</tr>
<tr>
<td>55</td>
<td>Produces long and complicated sentences such as: &quot;When we went to the park I had a go on the swings&quot;; &quot;I saw this man standing on the corner&quot;</td>
</tr>
<tr>
<td>56</td>
<td>Makes good use of gestures to get his/her meaning across</td>
</tr>
<tr>
<td>57</td>
<td>Shows concern when other people are upset</td>
</tr>
<tr>
<td>58</td>
<td>Speaks fluently and clearly, producing all speech sounds accurately and without any hesitation</td>
</tr>
<tr>
<td>59</td>
<td>Keeps quiet in situations where someone else is trying to talk or concentrate (e.g. when someone else is watching TV, or during formal occasions such as school assembly or a religious ceremony)</td>
</tr>
<tr>
<td>60</td>
<td>Realises the need to be polite - would pretend to be pleased if given a present s/he did not really like, and would avoid making personal comments about strangers</td>
</tr>
<tr>
<td>61</td>
<td>When answering a question, provides enough information without being over-precise</td>
</tr>
<tr>
<td>62</td>
<td>You can have an enjoyable, interesting conversation with him/her</td>
</tr>
<tr>
<td>63</td>
<td>Shows flexibility in adapting to unexpected situations: e.g. does not get upset if s/he planned to play on the computer, but has to do something else because it isn't working</td>
</tr>
<tr>
<td>64</td>
<td>Uses abstract words that refer to general concepts rather than something you can see - e.g. &quot;knowledge&quot;, &quot;politics&quot;, &quot;courage&quot;</td>
</tr>
<tr>
<td>65</td>
<td>Smiles appropriately when talking to people</td>
</tr>
<tr>
<td>66</td>
<td>Uses words that refer to whole classes of objects, rather than a specific item. E.g. refers to a table, chair and drawers as &quot;furniture&quot;, or to apples, bananas and pears as &quot;fruit&quot;</td>
</tr>
<tr>
<td>67</td>
<td>Talks about his/her friends; shows interest in what they do and say</td>
</tr>
<tr>
<td>68</td>
<td>Explains a past event (e.g. what s/he did at school, or what happened at a football game) clearly</td>
</tr>
<tr>
<td>69</td>
<td>Produces sentences containing &quot;because&quot; such as &quot;John had a cake because it was his birthday&quot;</td>
</tr>
<tr>
<td>70</td>
<td>Talks to others about their interests, rather than his/her own</td>
</tr>
</tbody>
</table>
REPETITIVE BEHAVIOR SCALE – Revised (RBS-R)

Name: ___________________________ ID#: ___________________________

Gender: female  male  Date of Birth: ___/___/_______  Today’s Date: ___/___/_______

Informant’s Name: ___________________________

Instructions:
Please rate this person’s behavior by reading each of the items listed and then choosing the score that best describes how much of a problem the item is for the person. Be sure to read and score all items listed. Make your ratings based on your observations and interactions with the person over the last month. Use the definitions in the box given below to score each item.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Behavior does not occur</td>
</tr>
<tr>
<td>1</td>
<td>Behavior occurs and is a mild problem</td>
</tr>
<tr>
<td>2</td>
<td>Behavior occurs and is a moderate problem</td>
</tr>
<tr>
<td>3</td>
<td>Behavior occurs and is a severe problem</td>
</tr>
</tbody>
</table>

When deciding on a score for each item, consider: (a) how frequently the behavior occurs (e.g., weekly versus hourly), (b) how difficult it is to interrupt the behavior (e.g., can be easily redirected versus becomes distressed if interrupted) and (c) how much the behavior interferes with ongoing events (e.g., easy to ignore or overlook versus very disruptive).

I. Stereotyped Behavior Subscale

(DEFINITION: apparently purposeless movements or actions that are repeated in a similar manner)

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 WHOLE BODY</td>
<td>(Body rocking, Body swaying)</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>2 HEAD</td>
<td>(Rolls Head, Nods Head, Turns Head)</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>3 HAND/FINGER</td>
<td>(Flaps hands, Wiggles or flicks fingers, Claps hands, Waves or shakes hand or arm)</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>4 LOCOMOTION</td>
<td>(Turns in circle(s), Whirls, Jumps, Bounces)</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>5 OBJECT USAGE</td>
<td>(Spins or twirls objects, Twiddles or slaps or throws objects, Lets objects fall out of hands)</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>6 SENSORY</td>
<td>(Covers eyes, Looks closely or gazes at hands or objects, Covers ears, smells or sniffs items, Rubs surfaces)</td>
<td>0 1 2 3</td>
</tr>
</tbody>
</table>
REPEETITIVE BEHAVIOR SCALE - Revised

0 = behavior does not occur
1 = behavior occurs and is a mild problem
2 = behavior occurs and is a moderate problem
3 = behavior occurs and is a severe problem

II. Self-Injurious Behavior Subscale

(DEFINITION: movement or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner)

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>HITS SELF WITH BODY PART (Hits or slaps head, face, or other body area)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>HITS SELF AGAINST SURFACE OR OBJECT (Hits or bangs head or other body part on table, floor or other surface)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>HITS SELF WITH OBJECT (Hits or bangs head or other body area with objects)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>BITES SELF (Bites hand, wrist, arm, lips or tongue)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>PULLS (Pulls hair or skin)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>RUBS OR SCRATCHES SELF (Rubs or scratches marks on arms, leg, face or torso)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>INSERTS FINGER OR OBJECT (Eye-poking, Ear-poking)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>SKIN PICKING (Picks at skin on face, hands, arms, legs or torso)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

III. Compulsive Behavior Subscale

(DEFINITION: behavior that is repeated and is performed according to a rule, or involves things being done "just so")

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>ARRANGING / ORDERING (Arranges certain objects in a particular pattern or place; Need for things to be even or symmetrical)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>COMPLETENESS (Must have doors opened or closed; Takes all items out of a container or area)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>WASHING / CLEANING (Excessively cleans certain body parts, Picks at lint or loose threads)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>CHECKING (Repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc.)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>COUNTING (Counts items or objects; Counts to a certain number or in a certain way)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>HOARDING / SAVING (Collects, hoards or hides specific items)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>REPEATING (Need to repeat routine events; In / out door, up / down from chair, clothing on/off)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>TOUCH / TAP (Need to touch, tap, or rub items, surfaces, or people)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
### IV. Ritualistic Behavior Subscale

**DEFINITION:** performing activities of daily living in a similar manner

| 23 | EATING / MEALTIME | (Strongly prefers/insists on eating/drinking only certain things; Eats or drinks items in a set order; Insists that meal related items are arranged in a certain way) | 0 | 1 | 2 | 3 |
| 24 | SLEEPING / BEDTIME | (Insists on certain pre-bedtime routines; Arranges items in room "just so" prior to bedtime; Insists that certain items be present with him/her during sleep; Insists that another person be present prior to or during sleep) | 0 | 1 | 2 | 3 |
| 25 | SELF-CARE — BATHROOM AND DRESSING | (Insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; Arranges items in a certain way in the bathroom or insists that bathroom items not be moved; Insists on wearing certain clothing items) | 0 | 1 | 2 | 3 |
| 26 | TRAVEL / TRANSPORTATION | (Insists on taking certain routes/paths; Must sit in specific location in vehicles; Insists that certain items be present during travel, e.g., toy or material; Insists on seeing or touching certain things or places during travel such as a sign or store) | 0 | 1 | 2 | 3 |
| 27 | PLAY / LEISURE | (Insists on certain play activities; Follows a rigid routine during play / leisure; Insists that certain items be present/available during play/leisure; Insists that other persons do certain things during play) | 0 | 1 | 2 | 3 |
| 28 | COMMUNICATION / SOCIAL INTERACTIONS | (Repeats same topic(s) during social interactions; Repetitive questioning; Insists on certain topics of conversation; Insists that others say certain things or respond in certain ways during interactions) | 0 | 1 | 2 | 3 |

### V. Sameness Behavior Subscale

**DEFINITION:** resistance to change, insisting that things stay the same

| 29 | Insists that things remain in the same place(s) (e.g., toys, supplies, furniture, pictures, etc.) | 0 | 1 | 2 | 3 |
| 30 | Objects to visiting new places | 0 | 1 | 2 | 3 |
| 31 | Becomes upset if interrupted in what he/she is doing | 0 | 1 | 2 | 3 |
| 32 | Insists on walking in a particular pattern (e.g., straight line) | 0 | 1 | 2 | 3 |
| 33 | Insists on sitting at the same place | 0 | 1 | 2 | 3 |
| 34 | Dislikes changes in appearance or behavior of the people around him/her | 0 | 1 | 2 | 3 |
| 35 | Insists on using a particular door | 0 | 1 | 2 | 3 |
| 36 | Likes the same CD, tape, record or piece of music played continually; Likes same movie / video or part of movie / video | 0 | 1 | 2 | 3 |
| 37 | Resists changing activities; Difficulty with transitions | 0 | 1 | 2 | 3 |
| 38 | Insists on same routine, household, school or work schedule everyday | 0 | 1 | 2 | 3 |
| 39 | Insists that specific things take place at specific times | 0 | 1 | 2 | 3 |
VI. Restricted Behavior Subscale

(DEFINITION: Limited range of focus, interest or activity)

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Rating 0</th>
<th>Rating 1</th>
<th>Rating 2</th>
<th>Rating 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Fascination, preoccupation with one subject or activity (e.g., trains, computers, weather, dinosaurs)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>41</td>
<td>Strongly attached to one specific object</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
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<td>42</td>
<td>Preoccupation with part(s) of object rather than the whole object (e.g., buttons on clothes, wheels on toy cars)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>43</td>
<td>Fascination, preoccupation with movement / things that move (e.g., fans, clocks)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Scoring Summary:

1. **Number of subscale items endorsed**: number of items in a subscale rated 1, 2, or 3
2. **Total subscale score**: sum of the ratings for all of the items in a subscale
3. **Overall number of items endorsed**: sum of the "Number of subscale items endorsed"
4. **Overall score**: sum of the "Total subscale scores"

<table>
<thead>
<tr>
<th>Subscale</th>
<th>Number of subscale items endorsed</th>
<th>Total subscale score</th>
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</thead>
<tbody>
<tr>
<td>I. Stereotyped Behavior</td>
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<td>II. Self-injurious Behavior</td>
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<td>III. Compulsive Behavior</td>
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<td>IV. Ritualistic Behavior</td>
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<td>V. Sameness Behavior</td>
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<tr>
<td>VI. Restricted Behavior</td>
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</tbody>
</table>

References for RBS-R:

RBS-R  Page - 4
Many of the following behaviours may not apply to the child or teenager in your care. For each item that does describe the person in your care, now or within the past six months, please circle the 2 if the item is very true or often true. Circle 1 if the item is somewhat or sometimes true of your child. If the item is not true of your child circle the 0.

0 = not true as far as you know  1 = somewhat or sometimes true  2 = very true or often true

If your child is unable to perform an item, circle the 0. For example, if your child has no speech, then for the item "Talks too much or too fast" circle the 0.

Underline any you are particularly concerned about

<table>
<thead>
<tr>
<th>Office Use Only</th>
<th>Please Circle</th>
<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>0 1 2</td>
<td>Appears depressed, downcast or unhappy</td>
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<td>2.</td>
<td>0 1 2</td>
<td>Avoids eye contact. Won't look you straight in the eye.</td>
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<td>3.</td>
<td>0 1 2</td>
<td>Aloof, in his/her own world.</td>
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<td>4.</td>
<td>0 1 2</td>
<td>Abusive. Swears at others.</td>
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<td>5.</td>
<td>0 1 2</td>
<td>Arranges objects or routine in a strict order. Please describe:</td>
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<td>6.</td>
<td>0 1 2</td>
<td>Bangs head.</td>
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<td>7.</td>
<td>0 1 2</td>
<td>Becomes over-excited.</td>
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<td>8.</td>
<td>0 1 2</td>
<td>Bites others.</td>
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<td>9.</td>
<td>0 1 2</td>
<td>Cannot attend to one activity for any length of time, poor attention span.</td>
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<td>10.</td>
<td>0 1 2</td>
<td>Chews or-mouths objects, or body parts.</td>
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<td>11.</td>
<td>0 1 2</td>
<td>Cries easily for no reason, or over small upsets.</td>
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<td>12.</td>
<td>0 1 2</td>
<td>Covers ears or is distressed when hears particular sounds. Please describe:</td>
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<tr>
<td>13.</td>
<td>0 1 2</td>
<td>Confuses the use of pronouns e.g. uses &quot;you&quot; instead of &quot;I&quot;.</td>
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<td>14.</td>
<td>0 1 2</td>
<td>Deliberately runs away.</td>
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<td>15.</td>
<td>0 1 2</td>
<td>Delusions: has a firmly held belief or idea that can't possibly be true. Please describe:</td>
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<td>16.</td>
<td>0 1 2</td>
<td>Distressed about being alone.</td>
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<td>17.</td>
<td>0 1 2</td>
<td>Doesn't show affection.</td>
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<td>18.</td>
<td>0 1 2</td>
<td>Doesn't respond to others' feelings, e.g. shows no response if a family member is crying.</td>
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<td>19.</td>
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<td>Easily distracted from his/her task, e.g. by noises.</td>
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<td>20.</td>
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<td>Easily led by others.</td>
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<td>21.</td>
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<td>Eats non-food items e.g. dirt, grass, soap.</td>
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<td>22.</td>
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<td>Excessively distressed if separated from familiar person.</td>
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<td>23.</td>
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<td>Fears particular things or situations, e.g. the dark or insects. Please describe:</td>
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<td>Facial twitches or grimaces.</td>
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<td>25.</td>
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<td>Flicks, taps, twirls objects repeatedly.</td>
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<td>Fussy eater or has food fads.</td>
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<td>27.</td>
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<td>Gorges food. Will do anything to get food e.g. takes food out of garbage bins or steals food.</td>
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<td>28.</td>
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<td>Gets obsessed with an idea or activity. Please describe:</td>
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<td>29.</td>
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<td>Grinds teeth.</td>
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<td>30.</td>
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<td>Has nightmares, night terrors or walks in sleep.</td>
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Please be sure you have answered all items

Continue next page ➔
0 = not true as far as you know  1 = somewhat or sometimes true  2 = very true or often true
Underline any you are particularly concerned about

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193
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Sleeps too little. Disrupted sleep.

Sleeps too much.

Seeks in whispers, high pitched voice, or other unusual tone or rhythm.

Switches lights on and off, pours water over and over; or similar repetitive activity.

Please describe:

Seeks, hears, something which isn't there. Hallucinations.

Please describe:

Talks about suicide.

Tense, anxious, worried.

Tells lies.

Tells or jumbled together with meaning difficult to follow.

Tries to manipulate or provoke others.

Underreacts to pain.

Unrealistically happy or elated.

Unusual body movements, posture, or way of walking.

Please describe:

Upset and distressed over small changes in routine or environment.

Please describe:

Urinates outside toilet, although toilet trained.

Very bossy.

Wanders aimlessly.

Whines or complains a lot.

Please write in any problems your child has that were not listed above.

Overall, do you feel your child has problems with feelings or behaviour, in addition to problems with development? If not, please circle the 0. If so, but they're minor, please circle the 1. If they're major problems, please circle the 2.

Please be sure you have answered all items

Are there any other comments you would like to make?

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THANK YOU

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<thead>
<tr>
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Author/s:
Villagonzalo, Kristi-Ann

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Redox biology and autism

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