Trial Protocol

Lay Title

FAB Trial - Fluoxetine for Autistic Behaviours

Scientific Title

Multi-site randomised controlled trial of Fluoxetine versus placebo for the treatment of restricted, repetitive and stereotyped behaviours in children and adolescents with autism

Chief Investigators: Professor Dinah Reddihough Dr Catherine Marraffa, Dr Roshan Virasinghe, Professor Philip Hazell, Associate Professor Michael Kohn, Dr John Wray, Dr Katherine Lee.

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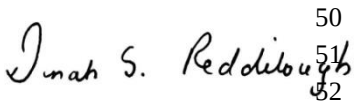
The Royal Children's Hospital Melbourne

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

AGREEMENT

This document is confidential. The Chief Investigator declares that they have read the final study protocol and any amendments. The Investigator will conduct the study according to the procedures specified in the study protocol, and in accordance with the Note for Guidance on Good Clinical Practice



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Chief Investigator Date

INDEX **PAGE**

84

INDEX **PAGE**

85

STUDY SYNOPSIS

Protocol Version: Version 1

Protocol Title:
Multi-site randomised controlled trial of Fluoxetine versus placebo for the treatment of restricted, repetitive and stereotyped behaviours in children and adolescents with autism.

Chief Investigators:
Professor Dinah Reddihough Dr Catherine Marraffa, Dr Roshan Virasinghe, Professor Philip Hazell, A/Prof Michael Kohn, Dr John Wray, Dr Katherine Lee.

Study Aim:
To determine the efficacy and safety of Fluoxetine in the treatment of restricted, repetitive and stereotyped behaviours in children and adolescents with autism compared to placebo.

Study Population:
Children and adolescents aged between 7.5 and less than 18 years with an autism spectrum disorder, and associated restricted, repetitive and stereotyped behaviours causing functional impairment.

Study Sites:
- Royal Children's Hospital (Victoria)
- Children's Hospital at Westmead (New South Wales)
- State Child Development Centre (Western Australia).

Study Design:
Randomised double-blind placebo-controlled trial with parallel group design. The study consists of a treatment period of 16 weeks, followed by weaning of the study medication (active or placebo) over 4 weeks, and final follow-up 2 weeks after the weaning is complete.

Study Medication:
Fluoxetine or placebo syrup, both taken orally once daily in the morning.

Dosage:
Fluoxetine or placebo syrup will be administered orally, once daily, in the morning. The study medication (fluoxetine or placebo) will be commenced at either 4 or 8 mg/day for the first week depending on the participant's weight (4mg if <40kg; 8mg if ≥40kg). The medication will then be titrated up at weekly intervals, for the next 3 weeks, using a flexible titration schedule (see page 12 for details). The maximum dose used will be 30mg/day for participants ≥ 40kg, and 20mg/day for participants < 40kg. No further dose increases will occur after week 4 of the trial. The dose may be decreased (or the medication ceased) at any time during the trial, should significant side effects occur, or if the doctor decides that it is no longer in the child's best interests to remain on the study drug. The duration of treatment will be 16 weeks, followed by weaning over a 4 week period. Side effects will be monitored throughout, and at 2 weeks post weaning off the study medication.

Number of Subjects:
146 subjects randomised into two groups (active and placebo).

SECTION 1: INTRODUCTION AND BACKGROUND

Introduction

The autism spectrum disorders (ASD) constitute a significant health disability burden, both within Australia and worldwide. In Australia a prevalence of 1 in 160 has been documented,[1] indicating that autism is a common disorder in our community.

The ASDs are neurodevelopmental disorders that cause lifelong and characteristic impairments in communication and social relatedness, and a pattern of restricted, repetitive and stereotyped interests and behaviours. The ASDs include the Diagnostic and Statistical Manual (DSM-IV TR) diagnoses of Autistic Disorder, Asperger's Disorder and Pervasive Developmental Disorder-Not Otherwise Specified.[2] The severity of the core features of autism vary significantly between individuals with an ASD. Variability is a hallmark of ASD, and extends to the presence of associated symptoms such as anxiety, irritability, aggression, and self-injury. Many individuals diagnosed with an ASD require lifelong support. As a consequence, there is a significant burden of care imposed on parents and families.

Repetitive behaviours constitute a core feature of autism, and are broadly defined as non-functional activities or interests that occur regularly and interfere with daily functioning at home, school, and in social settings. Repetitive behaviours include restricted and repetitive use of language, circumscribed interests and preoccupations, ritualistic behaviours, compulsive behaviours, stereotypic motor movements, repetitive non-functional use of objects, repetitive self-injury, unusual sensory interests (such as sniffing, mouthing, and licking), and difficulty coping with change.[3] Repetitive behaviours and their associated symptoms of anxiety, irritability, aggression and self-injury can have significant ramifications for both the child and family.

Published reports indicate that more than half of children and adolescents with autism are prescribed psychotropic medication. Antidepressants (mostly SSRIs) are the most commonly prescribed, with 21% to 32% of children with autism prescribed an antidepressant medication.[4-6] It is likely that SSRI agents are prescribed in this population for a number of indications, including mood disorders, anxiety, co-morbid obsessive-compulsive disorder (OCD), and troublesome repetitive behaviours. However the evidence for the use of SSRI medication for repetitive behaviours is inconclusive. In particular Therapeutic Goods Administration (TGA) in Australia, and the Food and Drug Administration (FDA) in the United States are yet to approve the use of SSRIs for repetitive behaviours in autism.

There are a number of SSRI agents licensed for use in Australia, including fluoxetine and fluvoxamine and in general they have similar efficacy and side effect profiles, but differing dosages. The use of standard recommended doses of SSRI medication in children with autism is now being questioned by experts in the field of paediatric psychopharmacology, with clinical experience suggesting that this population responds best to low doses, thereby minimising the risk of side effects, particularly 'behavioural activation'. When SSRIs are used in higher doses in children with autism, studies have shown behavioural activation to be a critical factor in drug tolerability.[7,8] The term "behavioural activation" refers to side effects such as insomnia, motor hyperactivity, agitation, aggression, irritability and anxiety. A randomised controlled crossover trial of fluoxetine for repetitive behaviours in 39 children with autism, found low-dose fluoxetine superior to placebo in the treatment of repetitive behaviours on the Children's Yale-Brown Obsessive Compulsive Scale – modified for pervasive developmental disorders. However, there was no significant improvement in global treatment response on the Clinical Global Impressions Scale. Low dose fluoxetine was well tolerated, with no significant difference in side effects between fluoxetine and placebo.[9]

Background

The mainstay of intervention in individuals with autism is individualised strategies to facilitate communication, socialisation, and behaviour management. However pharmacotherapy can also play a role in the management of targeted symptoms, with the aim of reducing interfering behaviours, such that the individual is more amenable to educational and behavioural interventions. These targeted symptoms include repetitive behaviours, anxiety, irritability, aggression, and self-injury.

Serotonin dysfunction in autism

There is evidence that serotonin plays a contributory role in the pathophysiology of autism, with converging support from genetic, biologic, and neuroimaging studies that higher levels of serotonin occur in some children with ASD. In particular, one study confirmed hyperserotinaemia in individuals with ASD, with approximately one-third of individuals having elevated platelet serotonin levels.[10] Depletion of the serotonin precursor tryptophan, has also been shown to induce a worsening of autistic symptoms in adults with autism.[11] In a study of 30 children with autism, less brain serotonin was produced on positron emission tomography (PET) imaging than in their non-autistic siblings (n=8) and children with epilepsy but without autism (n=16).[12]

Serotonin transporter gene and autism

The serotonin transporter gene (SLC6A4) is located on chromosome 17 and contains a variable repeat sequence in the promoter region. The gene has a more common 16 repeat long allele (L), and a less common 14 repeat short allele (S). Individuals with two long alleles (L/L genotypes) have been shown to rate more severely on the 'stereotyped and repetitive motor mannerisms' subdomain of the Autism Diagnostic Interview (ADI-R) than individuals with one or more short alleles.[13] A correlation between response to fluvoxamine and the serotonin transporter gene has also been described in a study involving 18 children with autism. Comparison of response to treatment and genotype or allele variation revealed that fluvoxamine tended to be more effective in individuals with an L/L or L/S genotype compared to the S/S genotype, and was significantly more effective in the 'L' allele variant than the 'S' allele variant.[14]

SSRI trials in autism

Due to the efficacy of SSRIs in treating obsessive-compulsive symptoms, and the fact that they address serotonin dysfunction, SSRIs have been receiving increasing attention as a potential treatment for the repetitive behaviours associated with autism. They act by inhibiting central nervous system neuronal reuptake of serotonin at the presynaptic nerve terminal, thereby increasing serotonin levels within the synapse. Several publications provide an overview of the use of SSRI medication in treating the core and associated symptoms of autism.[15,16] A recently completed Cochrane Review[17] (submitted) co-authored by two of our study investigators, examines the use of SSRIs in the treatment of ASD. This review identified six randomised placebo-controlled trials conducted in children and adults with autism, for a range of SSRI agents, with a cumulative total of 121 participants for all six trials.

- A 12-week double-blind placebo-controlled crossover trial of fluoxetine for repetitive behaviours in 39 children with autism, found low-dose fluoxetine (mean final dose of 9.9 ± 4.35mg/day) was superior to placebo in the treatment of repetitive behaviours on the Children's Yale-Brown Obsessive Compulsive Scale – modified for pervasive developmental disorders (CYBOCS-PDD). However there was no significant improvement in global treatment response on the Clinical Global Impressions Scale (CGI).[9]
- A 12-week double-blind placebo-controlled crossover trial of fluvoxamine in 18 children with autism found fluvoxamine to be effective in ten of the children (56%), based on the CGI (global treatment response).The CGI score was 7 for a very much improved condition and 1 for a very much worse condition. Patients with CGI scores indicating a very much improved condition

were classified as being excellent responders, and those who showed any improvements were classified as responders. From a clinical pint of view, five of the 18 (28%) cases were classified as excellent responders. In the case of those who exhibited minimal improvement, as estimated by CGI, fluvoxamine treatment was clinically effective in 10 of the 18 (56%) cases[14]

- A 16-week placebo-controlled crossover trial of fluoxetine in 6 adults with an ASD showed significant improvement in obsessions and anxiety with fluoxetine compared to placebo on the Yale-Brown Obsessive Compulsive Scale (YBOCS) – obsessions subscale and Hamilton Anxiety Scale.[18]
- A 12-week double-blind placebo-controlled randomised controlled trial evaluating fluvoxamine in 30 adults with an ASD found significant improvements in repetitive thoughts and behaviour, aggression, and global treatment response using the CGI, YBOCS, Vineland maladaptive behaviour subscales, and the Brown Aggression Scale in the fluvoxamine compared to placebo group.[19]
- A two-phase double-blind placebo-controlled crossover trial of fenfluramine in 15 children with autism found no evidence of a difference in behaviour between fenfluramine or placebo using the Ritvo-Freeman Real Life Rating Scale.[20]
- A 12-week double-blind placebo-controlled crossover trial of fenfluramine in 13 children with autism found no evidence of a difference between the treatment and placebo groups in the measured diagnostic features of autism using the Behaviour Summarised Evaluation Scale.[21]

The findings of these studies are inconclusive, with some showing evidence of effectiveness but not in others. All of these studies include small numbers of children, with large variation in the outcome measures, and a cross-over design used for all but one trial. The large variability in outcomes and heterogeneity in study design and populations makes it difficult to combine studies using a meta-analysis. Further trials with larger sample sizes, clearly defined outcome measures that are sensitive to change, and rigorous design are required to confirm the effectiveness of SSRIs.

Consultation with Clinical Experts

The opinions of the following clinical experts in the field of paediatric psycho-pharmacotherapy in autism were sought, in the planning and design of this research project:

- Professor Paramala J. Santosh
  Developmental Neuropsychiatrist
  Great Ormond Street Hospital for Children
  London, UK

- Dr David Dossetor
  Paediatric Psychiatrist, Area Director of Mental Health, Chair of the Division of Psychological, Developmental & Rehabilitation Medicine, and Head of the Centre for the Prevention of Psychological Problems in Children
  The Children's Hospital at Westmead

- Professor Alasdair Vance
  Academic Child Psychiatry Unit
  Royal Children's Hospital

Rationale for the study

Based on the current available literature, the efficacy of SSRIs for the treatment of restricted, repetitive and stereotyped behaviours in children with autism is yet to be established. Despite this, the 'off label' use of fluoxetine and other SSRIs in autism is increasingly common both in Australia and overseas. It is therefore of vital importance that high quality, controlled, and reproducible studies are performed to address the efficacy and safety of fluoxetine in children with autism.

There are a number of SSRI agents licensed for use in Australia, and in general they have similar efficacy and side effect profiles, but differing dosages. Fluoxetine is one of the most commonly prescribed SSRI agents in children with autism. Fluoxetine has also been studied in the largest controlled trial of an SSRI agent in children with autism to date. Therefore this study will investigate the use of fluoxetine, for repetitive behaviours in autism.

Dosage of fluoxetine

The appropriate dosing of SSRIs including fluoxetine in children with autism is still in question. 'Behavioural activation' may be a critical factor in drug tolerability in children when SSRIs are used in higher doses in children and adolescents. It is therefore of importance to determine the optimal dosing for children in this group.

Cook *et al*[7] reported an open trial of fluoxetine in 23 subjects (aged 7-28 years) with autism, for treatment of perseverative (repetitive) behaviours. The study utilised high doses of fluoxetine (final dose range 10-80mg/day). Six of the 23 subjects could not tolerate fluoxetine due to significant side effects, in particular 'behavioural activation' (including restlessness, hyperactivity, agitation, decreased appetite and insomnia).

Likewise, McDougle[8] described the results of a 12 week double-blind placebo-controlled study of another SSRI (fluvoxamine) in children (aged 5-18 years) with autism (McDougle *et al*, unpublished data). The mean dose utilised was 106.9 mg/day, with a range of 25-250mg/day. Fourteen of the 18 subjects receiving fluvoxamine experienced significant adverse events (insomnia, motor hyperactivity, agitation, aggression, irritability and anxiety). In contrast, a double-blind placebo-controlled study of fluvoxamine by the same authors[10], in 30 adults with autism, using a mean dose of 276.7 ± 41.7mg/day, and a dose range of 200-300mg/day found that fluvoxamine was well tolerated. Four patients reported nausea (3 in the treatment group, and 1 in the placebo group) in the first 2 weeks of treatment, which subsequently resolved. A further three patients experienced moderate sedation (2 in the treatment group, and 1 in the placebo group), which also resolved.

The use of low doses of SSRIs was explored in the only published randomised controlled trial of fluoxetine in children with autism to date. Hollander *et al* (2005)[9] performed a placebo-controlled crossover trial of low dose liquid fluoxetine for repetitive behaviours in autism, in children aged 5-17 years. The mean final dose was 9.9 +/- 4.35mg/day (0.36 +/- 0.116mg/kg/day), with a final dose range of 4.8-20mg/day. Importantly, with the use of low doses of fluoxetine, there was no significant difference between liquid fluoxetine and placebo in treatment emergent side effects. In addition, there were no trends or significant effect of drug versus placebo on the suicide subscale of the Overt Aggression Scale-Modified. Only one subject had suicidal ideation at any point during the trial, and this occurred while the subject was receiving placebo in the first phase.

The above studies highlight the importance of using lower doses, and titrating dose increases slowly, when treating children and adolescents with autism. We have therefore chosen to adopt a flexible dosing schedule for this study, starting at a low dose (4 mg/day for participants <40kg, and 8 mg/day for participants ≥40kg). The dose may subsequently be increased in small increments over 3 further steps (depending on drug tolerability/emergence of side effects and the clinical effect improvement seen in the child's repetitive behaviours). The maximum dose utilised will be 20 mg/day by week 6 (for participants <40kg), and 30 mg/day (for participants ≥40kg).

SECTION 2: METHOD

Aims

Primary Aim: To determine the efficacy of fluoxetine, compared to placebo, for reducing the frequency and severity of restricted, repetitive and stereotypic behaviours in children and adolescents with autism.

Hypothesis: Fluoxetine is effective compared to placebo in the treatment of restricted, repetitive and stereotypic behaviours in children and adolescents with autism compared to placebo.

Secondary Aim 1: To determine the frequency and type of adverse events reported for low dose fluoxetine compared to placebo, in children and adolescents with autism.

Secondary Aim 2: To provide exploratory data about whether there is a relationship between an individual's serotonin transporter genotype and response to treatment with fluoxetine compared to placebo in children and adolescents with autism.

Study Design

This study is a randomised double-blind placebo-controlled trial with a parallel group design. Treatment for both the active and control groups will be of 16 weeks duration. Participants will then be weaned off the study medication over 4 weeks and followed up for a further 2 weeks.

Trial Centres

The study will take place at 3 sites:

- Royal Children's Hospital (Victoria). This is the coordinating site.
- Children's Hospital at Westmead (New South Wales)
- State Child Development Centre (Western Australia).

Subjects – Numbers and Enrolment

Enrolment of 146 children and adolescents with an autism spectrum disorder will take place across 3 sites: Victoria, New South Wales and Western Australia.

- Trial investigators in each state will coordinate the recruitment of participants within their respective states. Participants will be recruited through paediatricians, child and adolescent psychiatrists and general practitioners throughout each state. Professionals in these states will be sent a letter explaining the study, along with a study synopsis and copies of the study brochure.
- Professionals that identify appropriate participants will be asked to assist recruitment by informing families about the study and providing a general introductory letter to the families about the study. In this letter there are details of how the families should proceed if they wish to participate. That is, they are invited to complete a tear-off slip at the bottom of the letter with the options to tick a box to receive further information regarding the study through a phone call from the study coordinator, or to decline involvement in the study. They will return this tear off slip in a reply paid envelope to the study coordinator in each state.
- Potential participants will also be informed of the study by mail through the Autism Victoria 'Research Participant Register' and the Western Australian Autism Register (where consent has been provided by the family to be contacted regarding research studies). The trial will also be widely advertised through the respective state autism and disability association websites and newsletters with potential participants invited to contact the study coordinators details in each state. Once contact is made, the letter described above will be dispatched.

Subjects -Definition of the Disorder

There are two international classification systems used for making the diagnosis of autism. The most commonly used for defining autism is the DSM IV TR (Diagnostic Statistical Manual IV-Text Revised). This classification will be used in this trial for subject selection.

DSM-IV TR (Diagnostic Statistical Manual IV- Text Revised).

Children much satisfy the criteria labelled A, B, and C. In addition, note the additional categories in criteria A that must also be satisfied.

A. A total of six (or more) items from (1), (2), and (3), with at least two items from (1), and one each from (2) and (3)

(**1**)**Qualitative impairment in social interaction, as manifested by at least two of the following:**

(a) Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction

(b) Failure to develop peer relationships appropriate to developmental level

(c) 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).

(d) Lack of social or emotional reciprocity

(**2**) **Qualitative impairments in communication as manifested by at least one of the following:**

(a) Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)

(b) In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others

(c) Stereotyped and repetitive use of language or idiosyncratic language

(d) lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

(**3**) **Restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:**

(a) Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus

(b) Apparently inflexible adherence to specific, nonfunctional routines or rituals

(c) Stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements)

(d) Persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play

C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.

Subjects - Inclusion criteria

- Males and females aged 7.5 to 18 years.
- A diagnosis of an Autism Spectrum Disorder based on the Autism Diagnostic Interview (ADI-R), and Diagnostic and Statistical Manual (DSM-IV TR) criteria for Autistic Disorder (as above), Asperger's Disorder and Pervasive Developmental Disorder – Not Otherwise Specified.
- Total score of ≥ 6 on the Children's Yale-Brown Obsessive Compulsive Scale – modified for pervasive developmental disorders (CYBOCS-PDD).
- Will be able to comply with the assessments and procedures required for the trial.

Subjects - Exclusion criteria

- A known DSM-IV diagnosis of Rett's Disorder, Childhood Disintegrative Disorder, Schizophrenia or Major Depression.
- Patients currently prescribed or who have received in the six week period prior to study entry:
  - ***Fluoxetine and other SSRI's***
  - Other psychotropic medications, including typical and atypical anti-psychotics, mood stabilisers and anxiolytics.
  - Atomoxetine
  - Monoamine oxidase inhibitor (MAOI) or pimozide
  - ***Antidepressants***
  - ***Use of St John's Wort***
- Co-morbid significant medical conditions (e.g. unstable seizure disorder, cardiac disease, liver failure or renal failure).
- Pregnancy –females of childbearing potential require a urine pregnancy test to exclude pregnancy.

Study Medication

Fluoxetine or placebo syrup will be administered orally, once a day in the morning (mane).

Study Medication - Formulation

The active and placebo medication will be produced by Richard Stenlake Chemists, specifically for this study. The active medication is fluoxetine syrup (2mg/mL), which contains fluoxetine hydrochloride dissolved in a methocel base (containing glycerine, polysorbate 80, sodium saccharin, citric acid, methocel E4M, sodium benzoate and water). The placebo medication is the methocel base only. Both the active and placebo medication are transparent red syrups with raspberry flavouring. The packaging for both the active and placebo syrups will be identical with containers labelled only with Study Syrup and the study ID number.

Study Medication - Dosage

- All dose adjustment decisions will be made by a study doctor.
- Fluoxetine or placebo syrup will be administered orally, once daily, in the morning (by the parent/guardian). The medication will be contained in a glass bottle. Parents/guardians will be advised to store at room temperature away from direct sunlight, and measure medication using a syringe (which will be provided by the clinical trials pharmacist).
- The study medication (fluoxetine or placebo) will be commenced at 4–8mg/day for the first week (4mg if <40Kg; 8mg if ≥40Kg). The medication will then be titrated up at weekly intervals, for the next 3 weeks***, using the titration schedule documented below (see Table A). Dosages will be increased according to the Clinical Global Impressions Scale which is a 7 point scale that requires the clinician to assess how much the patient's illness has improved or worsened relative to a baseline state at the beginning of the intervention and rated as: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse. If there is no change or the child's clinical state has improved, the dose will be increased as per the schedule below. If there are side effects the dose may be decreased (see below).***

  Parents will be issued with a diary card and will be instructed to fill this in during the telephone conversation. It will include the dose and time to be administered. The correct dose in mg and mls will be confirmed by the doctor undertaking the telephone conversation and this will be confirmed by email if possible. Parents will be asked to write this information on their diary card as a reminder about the dose.

At the conclusion of the first four weeks of the trial, parents will be asked to return the diary cards and these cards will be assessed for medication compliance. A new card will be commenced for the remainder of the study containing information on the current dose which should be maintained until week 16 unless instructed by the doctor. Any dose adjustments after this time should be detailed on the diary card.

- The maximum dose used will be 20mg/day for participants < 40kg and 30mg/day for participants ≥ 40kg. No further dose increases will occur after week 4 of the trial.
- The dose may be decreased (or the medication ceased) at any time during the trial, should significant side effects occur or if the doctor decides that it is no longer in the child's best interest to remain on the drug. ***These changes will be noted on the diary card.***
- The duration of treatment will be 16 weeks, followed by weaning over a 4 week period.
- At the completion of the 16 week treatment period, and following completion of the post trial assessments, both groups will be weaned off medication over a 4 week period.
- Each child will be monitored for a further 2 week after cessation of study medication to monitor side effects.

Table A: Summary of Dosing Schedule for Participants < 40 Kg

Week of Trial	**Dose (per day)**
1	4 mg
2	***8 mg***
3	***14 mg***
4	***20 mg***
5-16	Maintain effective dose
17-20	Wean off medication (at weekly intervals)

Table B: Summary of Dosing Schedule for Participants ≥ 40 Kg

Week of Trial	**Dose (per day)**
1	8 mg
2	***14 mg***
3	***22 mg***
4	***30 mg***
5-16	Maintain effective dose
17-20	Wean off medication (at weekly intervals)

Study Medication -Dispensation

Initially, one month's supply of medication will be dispensed for local participants and a maximum of two months' supply for families living a long distance from the study site. We will ask parents to collect a new supply of medication after one month but if this is too difficult for logistic reasons, the study coordinator will arrange for the medication to be posted. The amount of medication dispensed will be documented by the clinical trials pharmacist at each site. At the

completion of the trial, all medication bottles will be returned to the clinical trials pharmacist, and the amount of residual medication will be documented as a measure of adherence.

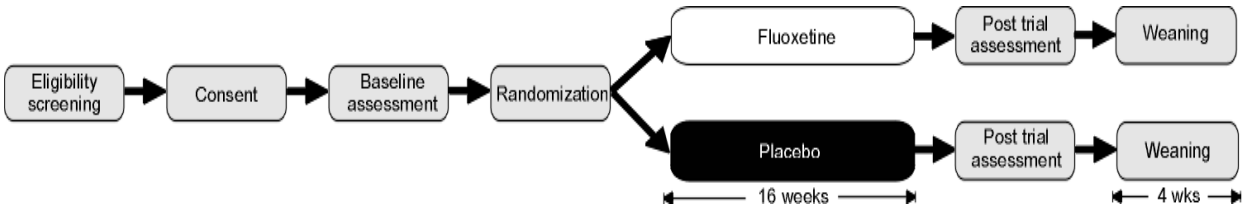
Excluded Medications

The use of other psychotropic medication, including typical and atypical anti-psychotics, antidepressants, mood stabilisers, anxiolytics and stimulant medication will not be permitted during the study period. ***The use of St John's Wort will not be permitted.***

Included Medications

If the child is already taking anticonvulsants, this medication will be continued but its use will be noted on the CRF.

Study Procedure



Initial discussion of study and eligibility screening - *phone (20min)*
Initial eligibility will be based on the inclusion and exclusion criteria defined above. Following receipt of the permission to be contacted, an initial phone call will be performed by the study coordinator in each state to firstly discuss the study and answer any questions that families might raise. If families are interested and willing to proceed, the study coordinator will then check eligibility criteria as follows:

- Does the child have an established diagnosis of an autism spectrum disorder and how was the diagnosis made?
- Age of child?
- Is the child currently on any medication?
- Does the child have any significant medical conditions?

If families meet the eligibility screening described above, and express willingness to receive further information, they will be sent the Parent/Guardian Information Sheet. The study coordinator will then make contact with them to establish that the Parent/Guardian Information Sheet has been received, to ascertain whether they are interested in proceeding and to answer any further questions. If families wish to further consider the study, an appointment will be made for the first visit for the pre-trial assessment.
Details of each phone call will be recorded whether or not the family decide to proceed with the study.

Pre-Trial Assessments – *Performed over approximately 3-4 study site visits*
Participants will be required to attend for 3-4 visits, to be completed over a 1-2 week period where possible. There will be some flexibility in the number of visits required depending on the ease of assessment of the child which may be influenced by behaviour and concentration. The following is a guide to the procedures and assessments undertaken at each visit.

Pre-Trial Visit 1 (conducted by study doctor)

- Some preliminary information on eligibility will have been sought at the telephone interview. These criteria, which have been listed previously, will be reviewed and confirmed at this first appointment.
- The study protocol will be discussed with families. Every effort will be made to ensure that families understand all aspects of the study and the demands that it will place upon them.
- Any questions raised by families will be answered.
- If a decision is reached that the participant is to enter the trial, informed consent will be obtained by the study doctor (see section on informed consent below).

Medical history and physical examination (see section on Case Report Form) will performed by a study doctor (1 hour). DNA collection for serotonin transporter gene polymorphism (SLC6A4) testing will be performed by cheek-brush and by saliva collection, by a study doctor. (20min) ***The purpose of the "Genetic Testing" is to determine whether there is a relationship between an individual's serotonin transporter genotype and response to treatment with fluoxetine compared to placebo in children and adolescents with autism (Secondary Aim 2 of the study). There are no***

insurance implications as future health is not affected. It will be optional in the study and parents will be given the choice as to whether to proceed with this or not to do so. Serotonin Transporter gene polymorphism (SLC6A4) testing will be performed at the Bruce Lefroy Centre, Murdoch Children's Research Institute. Sample collection will be by cheek brush and saliva collection using Oragene.DNA. Genotyping will be performed using a single PCR assay (Bio-Rad iCycler, California) and gel electrophoresis.

Pre-Trial Visit 2 (conducted by study psychologist)

- Baseline ratings will be obtained by a psychologist for the Children's Yale-Brown Obsessive Compulsion Scale – Modified for Pervasive Developmental Disorders (CYBOCS-PDD), Repetitive Behaviours Scale – Revised (RBS-R), Spence Children's Anxiety Scale (SCAC), Aberrant Behaviour Checklist (ABC) – Community Version, Clinical Global Impressions Scale (CGI) – Global Improvement and Efficacy Index (1 hour).
- For subjects who do not have previously documented cognitive testing, the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) or the Wechsler Non-Verbal Scale of Ability (WNV) will be administered by a psychologist (1½ hours). For the purposes of randomisation (see below), IQ as derived from the WISC-IV or WNV will be stratified into IQ < 70 (intellectual disability range) and IQ ≥ than 70 (average / borderline range).
- **Pre-Trial Visit 3 (conducted by study psychologist)**
- The diagnosis of an ASD will be confirmed by a clinical psychologist performing the Autism Diagnostic Interview (ADI-R) and by DSM-IV TR criteria on clinical interview of the parent/guardian (3 hours).

Families deemed ineligible

Details of all participants deemed ineligible will be recorded, and the particular reasons documented as to why they were deemed ineligible.

Randomisation Visit

Once all of the eligibility criteria have been checked and the child is deemed eligible for the study, the child will be allocated a study number and will be randomised to one of the two treatment arms (see section on randomisation). ***Initially, one month's supply of medication will be dispensed for local participants and a maximum of two months' supply for families living a long distance from the study site. We will ask parents to collect a new supply of medication after one month but if this is too difficult for logistic reasons, the study coordinator will arrange for the medication to be posted. All medication*** will be dispensed by the clinical trials pharmacists (see section on drug dispensing). ***The date on which the medication will be commenced will be decided and documented and this will be at the beginning of week 1 of the trial.***

Trial Week 1

Medication will be commenced (active drug or placebo).

Trial Week 2, 3 and 4

A phone consultation will be performed by the study doctor to document any adverse events and to consider a dose increase. The dose of the study drug to be given for the next week will be discussed and may be maintained or decreased, depending on the type and severity of any adverse effects experienced. The use of the Clinical Global Impressions Scale (CGI) will be used to determine whether there has been a change in clinical status (for description see below). Parents will be asked to write down the new dosage required ***(in mg and mls)*** to ensure that the correct dose is given to the child. ***The dose will be confirmed by email if possible.*** Details of the new dose will also be recorded on the patient's case report form (CRF).

At the conclusion of the first four weeks of the trial, parents will be asked to return the diary cards and these cards will be assessed for medication compliance. A new card will be commenced for the remainder of the study.

Trial Week 6, 8, 10, 12, 14 and 16

A phone consultation will be performed at Week 6, 8, 10, 12, 14, and 16 to document any adverse events. The minimum effective dose will be maintained between week 7 and 12, unless adverse events develop. Should adverse events occur, the dose may be maintained or decreased. This monitoring will be performed by the study research assistant in consultation with a study doctor.

Post-Trial Assessments

At the completion of the 16-week treatment period, the participant will be asked to return to the trials sites, where ratings will be obtained by a study psychologist for the CYBOCS-PDD, RBS-R, SCAS, ABC-community version and the CGI (1 hour).

Week 17-20

The study medication (active and placebo) will be weaned over 4 weeks, reducing the dose at weekly intervals, following the post-trial assessments. This will be performed by weekly phone calls by a study doctor. Any problems with adverse events will also be documented at weeks 18 and 20.

Week 22

The final follow up phone call will be made at Week 22, to document any problems with adverse events. This will be performed by the study research assistant in consultation with a study doctor.

Informed Consent

A 'Parent/guardian Information Statement and Consent Form' and 'Participant Information Statement and Consent Form' has been prepared. The parent/guardian's consent (and participants' signed consent, where the participant is deemed to be capable of giving consent as determined by the researcher) will be obtained. It will be the responsibility of the principal investigator at each site to ensure that the approved consent form has been signed by each parent (and the participant where appropriate) prior to entry into the study. Families will be advised that they are free to refuse to participate in, or to withdraw from the study at any time.

Randomisation

The study will include 146 children and adolescents who will be randomised between the fluoxetine and placebo groups. An independent statistician from the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Royal Children's Hospital will be responsible for generating the randomisation schedule. Block randomisation will be used, and stratified by site, age and IQ. There are three sites: The Royal Children's Hospital (Victoria), the Children's Hospital at Westmead (New South Wales) and the State Child Development Centre (Western Australia). Age will be stratified into 7.5 to <12 years and 12 to 18 years. IQ will be stratified into IQ ≥70 (average/borderline range) and IQ <70 (intellectual disability range). Thus there are a total of 12 strata (4 per site). The randomisation schedule for each site will be given to the clinical trials pharmacist at the site, who will then be responsible for arranging a sequential stock of trial medication for each stratum, labelled with only the study number, strata and instructions for use. This schedule will remain confidential. The independent statistician from CEBU will also keep a copy of the master randomisation schedule to check for any discrepancies. As each participant is enrolled in the study, they will be allocated the next ID code within the correct stratum.

Study Procedure Checklist

Procedure	Initial Screening	Testing [Pre]	Week1	Week2	Week3	Week 4	Weeks 6,8,10,12,14,16	Testing [Post]	Week 17-20	Week 22
Inc & Exc Criteria	❑									
Consent		❑								
Randomization		❑								
Trial Questionnaires		❑						❑		
IQ Testing		❑								
ADI-R		❑								
Medication Commenced			❑							
Dose Adjustments				❑	❑	❑				
Side Effects Checklist				❑	❑	❑	❑		❑	❑
Weaning of medication									❑	

Outcome Measures

Primary outcome (measured at baseline and 16 weeks)

Children's Yale-Brown Obsessive Compulsion Scale – Modified for Pervasive Developmental Disorders (CYBOCS-PDD): The CYBOCS[22] includes a detailed symptom checklist of possible obsessions and compulsions, which are then rated across five severity items (time spent on obsessions, interference, distress, resistance, and degree of control). The CYBOCS was modified for use in children with a pervasive developmental disorder by the RUPP Autism Network.[23] Both the CYBOCS-PDD and YBOCS (adult version) have been widely used in clinical drug trials in autism, and shown to be sensitive to change.[9,19] The CYBOCS-PDD is the primary outcome measure for this trial, in line with previous trials in this field.

Secondary outcomes (measured at baseline and 16 weeks)

Repetitive Behaviours Scale – Revised (RBS-R): This revised version of the scale captures the breadth of repetitive behaviours in autism. The RBS-R consists of six subscales: stereotypic behaviours, self injurious behaviours, compulsive behaviours, ritualistic behaviours, sameness behaviours, and restricted behaviours. Recently the RBS-R was validated in both children and adults with autism.[24]

Spence Children's Anxiety Scale (SCAC): This scale consists of a child and parent version.[25] The scale contains six subscales (panic/agoraphobia, social anxiety, separation anxiety, generalised anxiety, obsessions/compulsions, and fear of physical injury) and provides a total score. The validity and reliability of the Spence (child and parent versions) has been established for both anxiety-disordered and normal control populations.[26,27]

Aberrant Behaviour Checklist (ABC) – Community Version: The ABC assesses maladaptive behaviours in individuals with a developmental disability or intellectual impairment. The ABC items are grouped into five subscales: irritability/agitation, lethargy/social withdrawal, stereotypic behaviour, hyperactivity/non-compliance and inappropriate speech. The ABC has been widely used in clinical drug trials in autism. The factor structure of the ABC – community version has been found to be robust in an ASD sample of 275 individuals aged 3 to 21 years.[28]

Clinical Global Impressions Scale (CGI) – Global Improvement and Efficacy Index: The CGI[29] is a scale widely used to assess treatment response in psychiatric conditions. It is a three-item scale: severity of illness (rated on a 7-point scale), global improvement (rated on a 7-point scale), and efficacy index (rated on a 4-point scale). The CGI has been widely used in clinical drug trials in autism, and shown to be sensitive to change.[9,14,19]

Blinding

Parents/guardians, participants, and study investigators will remain blinded to treatment allocation until the final analysis is completed. The randomisation schedule will be known only to the independent statistician in CEBU and the clinical trials pharmacist at each site. In an emergency, a participant can be unblinded by a study doctor contacting the clinical trials pharmacist at that site. Should this occur, the chief investigator and Data Safety Monitoring Committee will also be notified with 24 hours.

Adverse Events

A medical history and physical examination will be documented prior to the commencement of the study, in order to assist in the interpretation of adverse events as they occur during the study.

All adverse experiences observed by the study doctor or reported by the subject/caregiver spontaneously or in response to a direct question during the study period (including up to 2 weeks after the last dose of study medication, will be evaluated by the investigator and noted in the adverse experience section of the patient's CRF. The nature of each experience, time of onset after drug administration, duration, severity and relationship to treatment should be established. Details of changes to the dosage schedule or any corrective treatment will be recorded in the CRF.

A rare but potentially serious adverse effect includes a short term increase in suicidal thoughts. In the very unlikely event that a child in the study is suspected of having suicidal thoughts during the study, the parents will be advised to contact one of the study team during office hours, or to take the child to a medical professional (e.g. GP or hospital emergency department) after hours for assessment. If this proves to be the case, the child will be discontinued from the trial, and ensure appropriate medical management and counselling will be sourced in conjunction with the family's health professionals.

For each adverse effect listed in the Trial Side Affect Checklist (and any other significant side effect which may be related to the medication, as deemed by a study doctor), maximum intensity will be assessed by the treating clinician and assigned one of the following:

- Mild - an adverse experience which is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

- Moderate - an adverse experience which is sufficiently discomforting to interfere with normal everyday activities.

- Severe - an adverse experience which is incapacitating and prevents normal everyday activities and/or requires therapeutic intervention (i.e. use of a prescription drug or hospitalisation).

Serious Adverse Events

Any serious adverse event occurring during the study period will be reported to the Principal Investigator at each site, and then by the Principal Investigator to Professor Dinah Reddihough, and the Human Research Ethics Committee and Adverse Drug Reaction Committee at each site, within 24 hours. In the event of a suspected serious adverse reaction, this should be reported to the TGA.

A *'Serious Adverse Event'* is defined as any event which is fatal, life-threatening, permanently disabling, incapacitating, results in hospitalisation, prolongs a hospital stay

'Life threatening' implies that the patient was at immediate risk of death from the event as it occurred. It does not include a reaction that, had it occurred in a more serious form, might have caused death.

'Permanent disability' indicates a permanent and substantial disruption of a patient's ability to carry out normal life functions.

Causality and its relationship, if any, to drug treatment, will be assessed and monitored for each adverse experience. Causality will be assessed using the following categories: - unrelated, remote, possible or probably as assessed by the treating clinician. The possible relationship of any adverse event to the study medication will be recorded on the Adverse Event Form.

The degree of certainty with which the relationship of an adverse experience is linked to drug treatment will be determined by how well the experience can be understood in terms of:

a) The known pharmacological properties of fluoxetine.

b) The knowledge of side effects of fluoxetine.
c) The course and nature of the adverse event.
d) The current health status of the patient.
e) The relationship between the time of administration and onset of adverse event, and of whether the adverse event can be reproduced on re-challenge of the drug.

Overdose

Any instance of overdose (suspected or confirmed) of the study drug will be communicated to the Principal Investigator at each site, and then by the Principal Investigator to Professor Dinah Reddihough, and the Human Research Ethics Committee and the Adverse Drug Reaction Committee at each site within 24 hours. Details will also be recorded on the participant's CRF.

Symptoms of overdose may include:

- Nausea and vomiting
- Unsteadiness or dizziness
- Nervousness, agitation or restlessness
- Confusion
- Uncontrollable shaking of a part of the body
- Hypomania
- Hallucinations
- Seizures
- Unresponsiveness
- Loss of consciousness

In the event of overdose causing significant medical symptoms as listed above, urgent medical attention should be sought. Possible measures required may include establishing and maintaining an airway, and ensuring adequate oxygenation and ventilation. Activated charcoal or gastric lavage may also be indicated.

In the event of families making a minor error in judgement of dose (less than 10% above the correct dose), this will be recorded on the CRF and any adverse effects noted.

Patient Completion and Withdrawal

Subjects will be considered to have completed the study upon completion of 16 weeks of the study medication. Premature termination of the study drug will be defined as completing less than 16 weeks of treatment. Even if the study drug is terminated, every effort will be made by the investigator to keep patients in the study until study completion. A patient may withdraw (or be withdrawn) from treatment prematurely for the following reasons:

- Significant adverse events (serious adverse event form must be completed)
- Patient/caregiver decision to discontinue the treatment
- Patient lost to follow-up

The reason for termination will be recorded on the patient completion/withdrawal section of the CRF. Every effort will be made to follow up patients who withdraw from treatment with drug-related adverse experiences, in order to determine the final outcome.

SECTION 3: MONITORING AND GOVERNANCE

Safety Monitoring

Monitoring for adverse events will occur weekly during tapering up and weaning down of the study medication, and at two weekly intervals at other times, up to 2 weeks after the completion of the study medication. All adverse events reported by the subject/caregiver spontaneously or in response a question from the study 'Side-effect Checklist', will be thoroughly evaluated by a study doctor by telephone, who will also ensure appropriate medical care is provided when adverse events are encountered. The nature of each adverse event, time of onset, duration, severity, and relationship to treatment will be established and recorded. All serious adverse events will be notified to the relevant Human Research Ethics Committee and Adverse Drug Reaction Committee, and well as the study Independent Data Safety Monitoring Committee (DSMC). Unexpected or previously unreported adverse events possibly related to medication use will also be reported to the TGA.

Independent Data Safety Monitoring Committee (DSMC)

An independent Data Safety Monitoring Committee (DSMC) will also be formed to monitor the safety of the study. The committee will consist of a Clinical Trials Statistician, a Paediatrician and a Child Psychiatrist, and will meet 6 monthly. At each DSMC meeting, the committee will be provided with a report of the recruitment, protocol violations and deviations, and adverse events to date will be presented according to (blinded) treatment arm. The report will be provided by the study statistician (Dr Katherine Lee) with study groups labelled as A and B to maintain the blinding. This report may be unblinded by an independent statistician if requested by the DSMC. The role of the DSMC is to monitor progress of the study, and in particular safety of the study to ensure that it remains ethical to continue recruitment. The DSMC will report to the Trial Management Group.

The DSMC report will include an analysis of the primary outcome, the score on the Children's Yale-Brown Obsessive Compulsion Scale – Modified for Pervasive Developmental Disorders (CYBOCS-PDD) at 16 weeks. The Peto stopping rule should be applied to the comparison of the primary outcome by treatment group, which is that the study be recommended to be stopped if the p-value for the comparison is <0.0001. There will be no formal stopping rule applied to the safety data.

Quality Assurance and Training

Trial psychologists will have the required experience and training to administer psychometric testing. They will also undertake training to obtain research accreditation for administration of the ADI-R. This can be undertaken at the Monash University Centre for Developmental Psychiatry and Psychology, in Victoria and at Annie's Centre in Randwick, New South Wales. Trial doctors (senior paediatric or psychiatry registrars) will be supervised by a chief investigator (paediatrician or psychiatrist) at each site.

SECTION 4: DATA MANAGEMENT

Database production, Entry of data, and Verification

A web based database will be developed for the trial, with data entry occurring at each site. Original Case Report Forms (CRF) will be used when entering information into the computer database, by the study coordinator at each site. CRFs will be photocopied. The photocopied CRF will be posted to the Study Coordinator at the RCH every 3 months, with the original version of the CRF remaining at the study site. Data will be checked against the original CRFs for accuracy by the Study Coordinator at the RCH, every 3 months. No investigation of the data will begin until an accurate database has been assured. All CRFs and data checking records will be retained as permanent records of the study.

Case Report Forms (CRF)

The CRF will be designed to collect all study data as described within this protocol. Each CRF will contain the patient's study number. The date of each consultation (including phone consultations) will be included on each form. All entries on the form will be neatly handwritten with a black ballpoint pen. The CRF will be commenced following successful screening of each subject, and kept up to date throughout the study. Errors on the CRF will be corrected by drawing a single line through the incorrect entry and writing in the correct value as close to the original as possible. The correction will then be initialled and dated by the authorised individual making the change. The original information on the form will not be obliterated, written over, or erased when making a correction.

Serotonin Transporter Gene

All samples will be de-identified prior to processing in the laboratory of Dr Paul Lockhart, Bruce Lefroy Centre, MCRI. The laboratory is located on the 10th floor of RCH. Screening will be by a cheek-brush sample (a 5.5mm cheek brush, Scrinet, Paris, France). Following sampling, the cheek brushes will be securely stored within the laboratory in the original packaging at 4℃ until extracted. A DNA lysate will be prepared using 50mM NaOH to lyse cells and 1M Tris-HCl (pH 7.5) to neutralise the lysate and stored at -20℃ in a locked freezer. The genotyping for the serotonin transporter gene promoter polymorphism involves a single PCR assay (Bio-Rad iCycler, California) and gel electrophoresis to simultaneously detect the wildtype and the variant allele. Samples will be held for 12 months for quality control and then destroyed. The results for the de-identified samples will be provided to the CI and also held for 12 months on a password protected file by the laboratory head.

SECTION 5: STATISTICAL METHODS

Primary outcome measure

- Total score on the CYBOCS-PDD at 16 weeks.

Secondary outcome measures

- RBS-R total score, and subscale scores at 16 weeks.
- SCAS total score at 16 weeks.
- ABC-community version total score and subscale scores at 16 weeks.
- CGI 'global improvement' and 'efficacy index' scores at 16 weeks.
- Frequency and type of adverse events across the 16 weeks of treatment and 4 weeks of weaning. and 2 weeks post completion of the study drug.

Serotonin transporter genotype will also be recorded.

Statistical Analysis

Analysis will be performed on an intention-to-treat basis including all participants with outcomes data available. Baseline characteristics will be presented separately for children in the active and placebo groups using means and standard deviations for continuous data (or medians and inter-quartile ranges for non-normal data) and proportions for categorical data. The primary outcome (total score on the CYBOCS-PDD at 16 weeks) will be compared for the active and placebo groups using unadjusted linear regression. Secondary outcomes will also be compared using unadjusted linear regression with proportions compared using unadjusted logistic regression. As a sensitivity analysis, regression models will also be fitted to the primary and secondary outcomes adjusted for pre-trial scores on the questionnaire of interest, age and IQ at baseline (as used in the randomisation) and any other baseline and demographic variables where an imbalance is found.

Sample Size and Power

The validation of the CYBOCS-PDD in 172 medication-free children with an ASD found a mean total score of 14.4 with a standard deviation of 3.86.[23] Based on the study investigators' clinical experience in the management of children with autism, a difference of 2 on the CYBOCS would represent a clinically important improvement in repetitive behaviours. Consequently, we aim to power our study to find an effect size of 0.5 (corresponding to a difference of 2 on the CYBOCS based on a standard deviation of 3.8). With 80% power and two-sided alpha of 0.05 this requires a sample size of 64 per treatment group. Allowing for a 15% drop-out rate, 73 participants will be required per treatment group. We therefore aim to recruit a total of 146 participants to this study.

SECTION 6: ADMINISTRATIVE PROCEDURE

Multisite Trial Coordination

The Study Coordinator will be based at the Royal Children's Hospital in Melbourne and will take responsibility for the overall coordination of the three trial sites. The Study Coordinator will work closely with Prof Reddihough (CI), Assoc Prof Kohn (PI) and Dr Wray (PI), who will be the study clinicians responsible for the coordination of the trial at each site. These four members along with Dr Katherine Lee make up the Trial Management Group. This group will meet 6 monthly soon after the DSMC meet and are responsible for decisions regarding the study.

The Study Coordinator and Prof Reddihough will meet once a fortnight to monitor the progress of the trial. Each state will have a research assistant and study clinician who will be responsible for the conduct of the trial in that state. In Victoria, the study coordinator will also undertake the role of research assistant. A teleconference will be held every 2 months between investigators at all three sites, to monitor the progress of the trial (including participant recruitment, clinical and safety issues, data collection/transfer, and logistics). The Study Coordinator will visit each site on a yearly basis (or 6 monthly if required) to ensure appropriate training, and to monitor adherence to procedures protocols.

Amendments to the Protocol / Deviations from the Protocol

All modifications of the study will be written and filed as amendments to this protocol, maintaining original section identification. Such modification(s) will be made jointly by the sponsor and the principal investigator, with the approval of the Ethics Committee. Any modifications to the study will be applied to all subsequent patients after Ethics Committee approval.

All deviations / violations to the protocol will be recorded. The person who identifies the deviation will report the event to the principal investigator. The deviation will be recorded in the participant's CRF, along with the reason for the deviation/violation. Where deviations to the protocol identify issues for protocol review, then the protocol will be amended as described above.

Early Termination of the Study

The study may be terminated prematurely if the number and severity of adverse events warrants this. The decision to terminate the study early will be made by the Trial Management Group in conjunction with the DSMC who will have access to the safety data during the study.

Drug Accountability

The Trial Pharmacist at each site will maintain adequate records of the receipt and dispensing of all study drugs supplied. Under no circumstances is the principal investigator or trial pharmacist to allow the study drug to be used other than as directed by the protocol. Any unused drug should be returned to the trial pharmacist at the conclusion of the study.

Drug Packaging, Labelling and Storage

The active and placebo medication will be produced by Richard Stenlake Chemists specifically for this study. Both the active medication (fluoxetine hydrochloride dissolved in a syrup at strength of 2mg/ml) and placebo (identical in appearance (red transparent syrup) and packaging (100ml or 200ml amber glass bottles). ***A form will be developed by the study team for the use of Richard Stenlake Chemists which will be used by them when issuing new supplies of medication. They will be asked to label medication as active medication (drug) or placebo. Each Centre will have a clinical trials pharmacist and this pharmacist will be responsible for dispensing the medication according to the randomization schedule developed by Clinical Epidemiology and Biostatistics.***

Labelling of the study medication will include the study number, strata, expiry date and instructions for use. The study medication (both active and placebo) will be stored in the Pharmacy Department at each site, in secured cool dry conditions at room temperature ***(or refrigerated) according to data from on-going stability testing by Stenlake Compounding Pharmacy.***

Retention of Study Documents

All documents (both paper and computer records) will be kept at least until the youngest participant turns 25 years, as specified in the Guidelines for Good Clinical Research Practice.

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APPENDIX 1:

Initials: _____ Study ID: _____

SIDE EFFECTS CHECKLIST

Date: ____/____/____

Form completed by: _____

Information obtained from:
- ❑ Mother
- ❑ Father
- ❑ Other primary caregiver (if so specify) _____

Interpreter used:
- ❑ No
- ❑ Yes

If yes, specify language: _____

If any of the side effects below are present, please fill in the details in the Non-Serious Adverse Events Form or Serious Adverse Events Form in the Case Report Form.
All Serious Adverse Reactions must be reported immediately to the Principal Investigator.

Monitoring for suicidal ideation

Mood change	❑
Irritability	❑
Thoughts of death/suicide	❑
Suicidal intent	❑
Suicide attempt	❑

Body as a whole

Asthenia	❑
Allergic reactions	❑
Anaphylactoid reaction	❑
Serotonin syndrome	❑
Chills	❑
Photosensitivity reaction	❑
Serum sickness	❑

Nervous system

Insomnia	❑
Somnolence	❑
Abnormal dreams	❑

Anxiety/Nervousness ❑

Tremor ❑

Headache ❑

Manic reaction ❑

Myoclonus ❑

Seizures ❑

Akathisia ❑

Ataxia ❑

Buccoglossal syndrome ❑

Depersonalisation ❑

Cardiovascular system

Palpitations ❑

Vasodilatation ❑

Orthostatic hypotension ❑

Digestive system

Dry mouth ❑

Anorexia ❑

Dyspepsia ❑

Nausea ❑

Vomiting ❑

Diarrhoea ❑

Weight loss ❑

Dysphagia ❑

Haematological system

Ecchymosis ❑

Musculoskeletal system

Twitching ❑

Respiratory system

Yawning ❑

Skin

Pruritis ❑

Rash ❑

Sweating ❑

Urticaria ❑

Alopecia ❑

Special senses

Abnormal vision ❑

Taste perversion ❑

Mydriasis ❑

Urogenital

Abnormal ejaculation ❑

Impotence ❑

Urinary frequency ❑

Priapism ❑

Any other side effects or problems: _____

_____

_____

_____

_____

APPENDIX 2:

Stability report from Stenlake Compounding Pharmacy

Technical Report 100709
July 2009

Fluoxetine Syrup Stability Testing

1. Summary

In June 2008, Stenlake Compounding was contracted by the Royal Children's Hospital in Melbourne, to develop a stable syrup formulation containing 2mg / ml fluoxetine (present as the hydrochloride salt) as the active. In addition data was required confirming the shelf life of the formulated syrup.

A stable formulation was developed for the active in July 2008.

Concurrently to developing the formulation, an analytical method (based on liquid chromatography) was also developed to

a) Confirm the active content of the syrup and
b) Enable testing of stored samples to define a definite shelf life for the product.

Samples of the prepared formulation were stored in amber and clear glass bottles under various conditions – refrigerated (8 deg), room temperature (stored on a laboratory benchtop) and heated (stored in a 40 deg oven).

Assay of the active fluoxetine content of the samples were carried out on a regular basis until a clear reduction in the activity level was noted.

The results from the study are shown in detail below, but from the data a **shelf life of 7 months can be safely assumed even at elevated temperatures.** It appears that protection from light (i.e. storage in amber bottles) will also assist in maintaining the stability of the formulation.

2. Sample Preparation and storage.

Test syrups were prepared on the 15/07/08 and the 30/07/2008. Portions of the syrup were transferred to a series of clean amber and clear glass bottles. One amber and one clear bottle were from the material prepared on the 17th were stored at room temperature and in a 40 deg oven. Samples from the batch prepared on the 30th were stored at room temperature and 40 degrees but also in a refrigerator (8 degrees).

On a regular basis, samples were extracted from some and occasionally all of the retained samples for assay of the active fluoxetine content.

A weighed amount of syrup sample was extracted from the sample containers directly into volumetric flasks. The samples were then diluted to volume with demineralised water and mixed well prior to analysis.

3. Analytical Methodology

Chromatographic analysis was carried out using a Waters Gradient HPLC system consisting of

- Waters 510 pumps
- Waters Automated Gradient Controller
- Waters 715 WISP Autosampler
- Waters 486 UV Detector

Initial UV work to determine the optimum wavelength for fluoxetine determination was carried out on a Varian DMS 80 Scanning UV/vis Spectrophotometer.

The analytical method was developed in house using modifications to published methods.

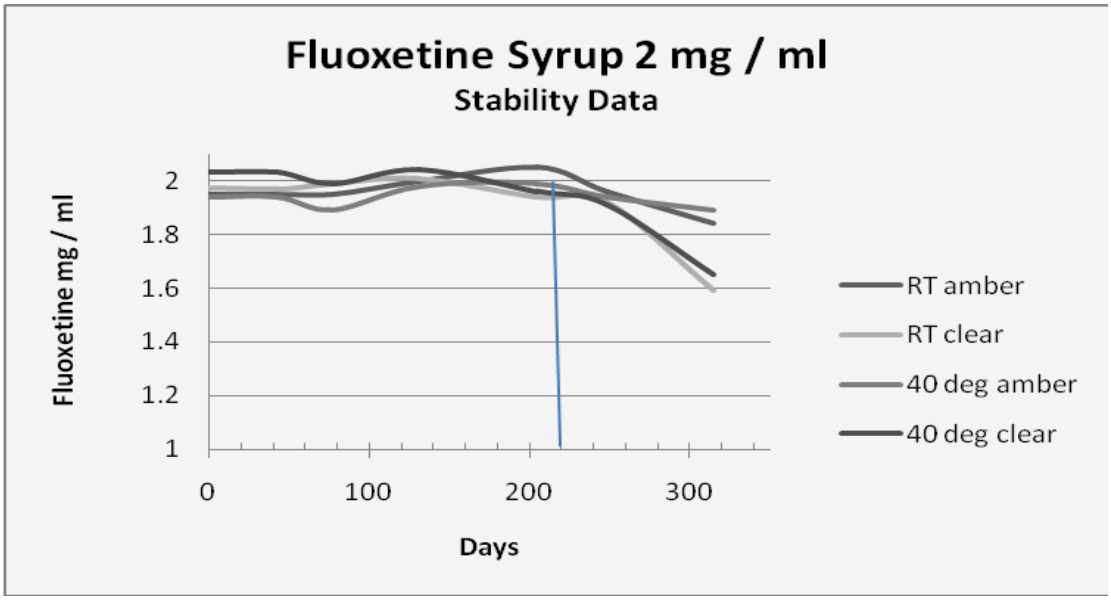
Column	:	Spherisorb CN-RP	
Mobile Phase	:	A	100% acetonitrile
		B	Triethanolamine buffer
Flow Rate	:		1 ml / minute
			45% A / 55% B

Internal standards were not utilised but the technique of standard additions was initially used to ensure the method did not suffer from interference when used to analyse the active syrup. Analysis by standard addition produced the same assay as direct analysis against aqueous standards.

4. Results

Test Date	Formulation Date	Container Type	Storage Temp	mg / ml fluoxetine HCL	mg / ml Fluoxetine	Days on Test
28/08/2008	15/07/2008	amber	40 deg	2.16	1.94	44
	15/07/2008	amber	RT	2.18	1.95	44
	15/07/2008	clear	40 deg	2.26	2.03	44
	15/07/2008	clear	RT	2.20	1.97	44
	30/07/2008	amber	40 deg	2.16	1.94	29
	30/07/2008	amber	8 deg	2.12	1.90	29
	30/07/2008	amber	RT	2.14	1.91	29
	30/07/2008	clear	40 deg	2.15	1.93	29
	30/07/2008	clear	8 deg	2.16	1.94	29
	30/07/2008	clear	RT	2.19	1.96	29
2/10/2008	15/07/2008	amber	40 deg	2.11	1.89	79
	15/07/2008	amber	RT	2.18	1.95	79
	15/07/2008	clear	40 deg	2.22	1.99	79
	15/07/2008	clear	RT	2.23	1.99	79
	30/07/2008	amber	40 deg	2.16	1.93	64
	30/07/2008	amber	8 deg	2.16	1.93	64
	30/07/2008	amber	RT	2.13	1.91	64
	30/07/2008	clear	40 deg	2.07	1.85	64
	30/07/2008	clear	8 deg	2.21	1.98	64
	30/07/2008	clear	RT	2.26	2.03	64
26/11/2008	15/07/2008	amber	40 deg	2.21	1.98	134
	15/07/2008	amber	RT	2.23	2.00	134
	15/07/2008	clear	40 deg	2.15	1.93	134
	15/07/2008	clear	RT	2.25	2.01	134
	30/07/2008	clear	40 deg	2.28	2.04	119
10/02/2009	15/07/2008	amber	40 deg	2.22	1.99	210
	15/07/2008	amber	RT	2.29	2.05	210
	15/07/2008	clear	40 deg	2.19	1.96	210
	15/07/2008	clear	RT	2.17	1.94	210
24/03/2009	15/07/2008	amber	40 deg	2.16	1.94	252
	15/07/2008	amber	RT	2.18	1.96	252
	15/07/2008	clear	40 deg	2.14	1.91	252
	15/07/2008	clear	RT	2.14	1.92	252
30/05/2009	15/07/2008	amber	40 deg	1.84	1.65	319
	15/07/2008	amber	RT	2.05	1.84	319
	15/07/2008	clear	40 deg	1.78	1.59	319
	15/07/2008	clear	RT	2.10	1.89	319

1354
1355



1356