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PhD thesis

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The effect of selective serotonin reuptake inhibitors in healthy first-degree relatives of patients with major depressive disorder

- an experimental medicine blinded controlled trial

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Not everything that counts can be counted,
and not everything that can be counted counts

(Albert Einstein, 1879-1955)

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Preface

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Ulla Benedichte Knorr

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Introduction

Depression is a common,[1-3], costly,[4] and recurrent disorder[5-7] that is associated with considerable morbidity[8] and excess mortality.[9] The pathogenesis of depression is unknown, however the serotonergic system has, among others, been suggested to play a major role in the pathogenesis of depression[10;11] and the effect of antidepressant treatment is well established.[12-14] Selective serotonin reuptake inhibitors (SSRI) are first-line pharmacological treatment options and 50% to 70% of patients respond to first treatment.[15] Further, antidepressant treatment is recommended for relapse prevention in depressive disorders.[16;17] However, the mechanisms, by which SSRIs act in depressed patients, remain widely unknown. In research of the mechanisms of the effect of antidepressant treatment, it has been difficult to differentiate, if changes were related to an effect of the antidepressant treatment or if changes were related to recovery from the depressive disorder per se. Experimental medicine in psychiatry supplements placebo controlled trials in depression. Thus, experimental medicine in psychiatry is research undertaken in human beings to identify mechanisms of pathology or disease, or to test the validity and importance of new discoveries or treatments, relating where appropriate to model systems.[18] Knowledge of the effect of antidepressant intervention might lead to a better understanding of the pathogenesis of the depressive disorder and could lead to the development of new strategies for treatment.

The depressive disorder is a familial disorder, and its familiarity mostly or entirely results from genetic influences.[19] Potential biomarkers for depression[20] such as 1) dysregulation of the hypothalamus-pituitary-adreno-cortical (HPA) axis[21-23], 2) the personality trait neuroticism[24;25] and 3) cognitive dysfunction[26-28] have also been detected in healthy first-degree relatives of patients with depressive disorder. This group of individuals represents the focus of interest in this thesis.

1.1. The effect of SSRI in healthy

A recent systematic review by Knorr and Kessing evaluated trials in which the effect of an intervention with a SSRI for 7 or more days in healthy participants was studied.[29] A total of 33 trials, investigating six different SSRIs and 163 outcome tests were identified. The findings were divergent which seemed to be a result of a number of methodological drawbacks. Few studies presented information on factors that may influence outcomes such as age, gender, ethnicity, family history of psychiatric disorder, drug levels, and none fulfilled modern principles of conducting and reporting randomised controlled trials. The review summarized that a relatively large number of statistically significant findings seemed to suggest a true effect on some outcome measures of SSRIs in healthy subjects, although most of these findings were rarely confirmed. The great variety of tests used made it difficult to integrate the individual investigation findings, thus conducting a meta-analysis was impossible. Specifically, the review concluded that no long-term (7 or more days) trial has investigated the effect of SSRIs in healthy subjects with a family history of MDD, which was the aim of the trial in the present thesis.

In the following, data will shortly be presented on the effect of SSRI on 1) the hypothalamus-pituitary-adreno-cortical (HPA) axis, 2) neuroticism and 3) cognitive function.

1.2. The effect of SSRI on hypothalamus-pituitary-adreno-cortical (HPA) axis regulation

Depression has been associated with an altered function of the HPA-axis,[30] including increased cortisol responses to the dexamethasone corticotropin releasing hormone (DEX-CRH) test.[31] Previous studies have shown that even healthy first-degree relatives to patients with major depressive disorder (MDD) may present with an abnormal HPA response to the DEX-CRH test, with an intermediary response when compared to healthy controls and patients with major depression[32] and, salivary cortisol have been shown to be increased in individuals with a family

history of MDD as compared to healthy individuals without a family history of MDD.[33-35] Several observations suggest that a disintegration of interactions between the serotonergic neurotransmitter system and the HPA neuroendocrine system may be present in patients with depression. The two systems may be connected; thus, single dose interventions of a selective serotonin re-uptake inhibitor (SSRI) increased serum corticosterone levels in normal rats[36;37] and plasma corticosteroid levels in healthy humans.[38-42] On the contrary, plasma levels of HPA-axis hormones, corticosterone and adrenocorticotropic hormone (ACTH), decreased after 15 days of intervention with citalopram in rats,[43] but the effect in healthy humans remains unknown.

1.3. The effect of SSRI on neuroticism

Neuroticism seems to reflect an enduring vulnerability to MDD.[44] This may partly reflect shared genetic risk factors and most of the genetic risk for MDD expressed via personality is captured by neuroticism, with a modest amount by conscientiousness, and small amounts by openness, extroversion, and agreeableness.[45;46] When neuroticism decreases in patients with depression who are treated with antidepressants, it has been difficult to clearly distinguish the treatment effect on neuroticism from the treatment effect on the depressive disorder, as remission of depressive symptoms is associated with partial normalization of neuroticism.[47] Decrease in neuroticism scores during paroxetine treatment of patients with MDD, even after controlling for depression improvement, has been observed in a large group of depressed patients.[48] Thus, it is possible that response to SSRIs may be mediated at least partly via a decrease in neuroticism.[49;50] Higher neuroticism has been associated with higher thalamic serotonin binding.[51] Furthermore, a recent study (partly from our group) has suggested that familial risk of depression and neuroticism interact in their relation to the degree of specific serotonin transporter binding.[52]

Two randomised trials have investigated the effect of SSRI on behaviour and aspects of personality with some relation to neuroticism in healthy participants without a family history of MDD. Thus, four weeks intervention with paroxetine 20 mg/day (n = 26) versus placebo (n = 25) significantly increased social affiliation and decreased negative affect.[53] Further, two weeks intervention with citalopram 20mg/day (n = 11) compared with placebo (n = 9) induced a statistically significant increase in self-directedness.[54] Furthermore, no effect of SSRI on depressive symptoms measured by Hamilton Depression Rating Scale[55] (HAM-D) in healthy individuals have been shown.[56-59] The results from these trials suggest that SSRI administration may affect personality even in the absence of clinical depression. Results from a number of studies, although not all[60] have suggested increased levels of neuroticism in healthy first-degree relatives of patients with MDD compared to healthy individuals without a family history of MDD.[61] However, no trial has investigated the effect of SSRIs on neuroticism and other personality dimensions in healthy individuals with a family history of MDD.[62]

1.4. The effect of SSRI on cognitive function

A wide range of cognitive deficits is a consistent finding in depression.[63] Cognitive function is a predictor of the functional and psychosocial burden of illness in MDD and consequently a pertinent candidate predictor of treatment response.[64] With recovery from MDD, abnormalities in cognitive function tend to normalize but cognitive impairment is seen both in recovered patients and in healthy first-degree relatives of patients with MDD.[65-67] The diversity of symptoms in MDD suggests that many areas of the brain are involved in the aetiology of the disorder. The serotonin transporter is expressed abundantly in the raphe nucleus and in the limbic system which may be the main site of action for SSRI.[68] It is, however, not clear whether treatment with SSRIs in patients with MDD results in a direct improvement of cognition or whether the effect of SSRIs on cognitive

function is secondary to the effect of SSRIs on depressive symptoms. A neuropsychological hypothesis of antidepressants drug action suggests that, at the neuropsychological level, antidepressants work by remediating negative affective biases in depression and anxiety and that these actions occur relatively quickly following drug administration.[69-71] The effect on cognitive function by long term intervention with a SSRI in healthy first-degree relatives of patients with MDD, has not yet been investigated.[72]

2. Aim and hypotheses

The aim of the present trial was to test the hypothesis that an intervention with a SSRI compared with placebo for first-degree relatives of patients with MDD:

1. Decreases the plasma cortisol response in the DEX-CRH test,
2. Decreases self-reported scores for the personality trait neuroticism,
3. Increases cognitive function.

3. Methods

3.1. Design

The AGENDA (associations between gene polymorphisms, endophenotypes for depression and antidepressive treatment) trial was designed as a participant, investigator, observer, and data-analyst-blinded randomised trial in which 80 participants were allocated to receive either escitalopram 10 mg/day or matching placebo for four weeks.

3.2. Approvals and registrations

The trial was approved by the Local Ethics Committee: H-KF 307413, The Danish Medicines Agency: 2612-3162 and the Danish Data Agency: 2006-41-6737. The trial was registered in EudraCT: 2006-001750-28 and at the ClinicalTrials.gov: NCT 00386841.

3.3. Study organization

The study was conducted from July 2007 until July 2009 at the Department of Psychiatry, Rigshospitalet, Copenhagen University Hospital, Denmark, as part of the Centre for Pharmacogenomics, University of Copenhagen. The trial had a data monitoring and safety committee (DMSC) that was independent of the investigators conducting the trial. The trial protocol was published ahead of trial completion.[73] The trial was conducted and monitored in accordance with the International Conference on Harmonization for Good Clinical Practice guidelines[74] and the Declaration of Helsinki 2002 (www.wma.net/e/policy/b3.htm).

3.4. Probands

Probands were patients with a diagnosis of MDD from psychiatric hospital in- or out-patient contact in Denmark who participated in ongoing studies at the Department of Psychiatry, Rigshospitalet, Denmark. Their diagnoses were validated by face-to-face interviews including the semi-structured interview Schedules for Clinical Assessment in Neuropsychiatry (SCAN)[75] by trained medical doctors. Probands were asked to permit a contact to their adult children and/or siblings who were the eligible participants for the AGENDA trial. The probands (n = 466) gave written permission to contact 359 first-degree relatives, whom were the potential participants in the trial.

3.5. Participants

Individuals of either sex, aged 18 – 60 with Danish ethnicity (defined as, born in Denmark, with Danish parents and European grandparents) were eligible for the trial. Ethnicity was used to get a genetically homogeneous sample. We excluded individuals with somatic illnesses or a handicap that made participation in the trial impossible while six individuals with stable, treated, milder medical conditions were included: hypertensio arterialis (three), pancreatitis antea (one), hypothyroidism (one), and acne vulgaris (one). Furthermore, we excluded individuals with a daily intake of drugs interfering with corticosteroids or escitalopram (cipralex), including birth control pills or any kind of corticosteroids, and individuals who were allergic to the study drug or placebo. Additionally, former medical or psychological treatment for diseases in the affective or schizophrenic spectrum and current abuse of alcohol or psychotropic medication led to exclusion. Women who were trying to conceive, or who were pregnant or breastfeeding were excluded. Women were preferably in the luteal phase of the menstrual cycle at the time of randomisation. Women taking birth control pills were instructed to discontinue these six weeks prior to entering the trial. Furthermore, all women were carefully instructed to use double barrier birth control methods and pregnancy tests were performed both before and after the intervention.[73]

3.6. Interventions

The participants were randomised to self-administer a single dose of either escitalopram 10 mg or matching placebo each evening for four weeks. Escitalopram and placebo tablets were identical in appearance, colour, smell, and solubility allowing for blinding of the assignment to intervention or placebo. H. Lundbeck A/S provided identically appearing blister packages containing escitalopram or placebo. An independent pharmacist then packed, sealed, and numbered the drug packages according to a randomisation list provided and concealed by the Copenhagen Trial Unit (CTU). On

completion of four weeks of double-blind intervention participants entered a five-day blinded down-titration period to nil medication. Adherence to the protocol was sought by making weekly telephone calls to the enrolled participants. The participants were asked at the end of the trial, how adherent they had been to the protocol, and if they had missed taking any tablets.

3.7. Randomisation

Randomisation to one of the two intervention groups was done immediately after it had been established that a participant fulfilled all the inclusion criteria and none of the exclusion criteria. CTU performed the centralized computerized randomisation by telephone to secure adequate allocation sequence generation and allocation concealment. Randomisation was stratified in blocks of six, by age (18–31 and 32–60 years) and sex. Only the data manager knew the block size. Participants were randomised in 1:1 to receive either escitalopram 10 mg or placebo.

3.8. Blinding

All trial personnel and participants were blinded to the packaging of the trial drug, and blinding was maintained throughout monitoring, follow-up, assessment of outcomes, data management, data analyses, and drawing the conclusions, thus in accordance with recommended suggestions.[76] At the assessment after four weeks intervention, each participant and the principal investigator (UK) made a guess as to which intervention the participant had received. The agreement between the actual intervention and the guesses was estimated to assess the degree to which blinding had been demasked, thus κ: < 0 no; 0.0-0.20 = slight; 0.21-0.40 = some; 0.41-0.60 = moderate; 0.61-0.80 = substantial; 0.81-1.00 = almost complete demasking.

3.9. Definition of outcomes

In a recent paper, Knorr and Kessing suggested the effect of SSRIs in healthy subjects with a family history of affective disorder as a new avenue of research for biomarkers and endophenotypes in depression.[77]

Depression has a wide range of possible features that can be measured and tested as possible endophenotypes.[78] The nature of this trial was experimental and since no prior trial has investigated the effect of SSRI on healthy first-degree relatives of patients with depression, we chose to include many outcomes.

3.10. Primary outcome

During selection of the primary outcome, it was stressed that the outcome should be objective and of clinical importance. Further, blinding should be possible at all levels of assessment and analyses. The measurement of the change in plasma cortisol in the combined DEX-CRH test from entry before to four weeks during intervention fulfils these criteria. Plasma cortisol was estimated as the total area under the curve (AUC-total) from administration of CRH at 15.00 to the last plasma cortisol measure at 18.00.[79]

3.10.1. The combined dexamethasone corticotropin releasing hormone test.

Cortisol and ACTH levels in response to the DEX-CRH test were measured before and after four weeks of intervention. The DEX-CRH test was performed according to international standards.[80] On both occasions the procedures were as follows: participants were given dexamethasone 1.5 mg orally at 23:00 the evening before the test. Participants were instructed to go to bed before midnight and to get up the following day between 6:00 and 8:00. They were instructed to have lunch at 12:00, to refrain from hard exercise, and beverages with caffeine. At 13:30 they arrived at the clinic.

Participants were resting supine (Figure 1) and were only allowed to leave the bed to use the bathroom. An indwelling intravenous catheter was inserted in an antecubital vein. During the test, participants did not eat, smoke, or drink, with the exception of small amounts of water but they were allowed to read and listen to the radio. At 15:00, 100 µg human CRH (Corticorelin Human triflutat, Kiel, Ferring) reconstituted in 1 ml water, sodium chloride and hydrochloric acid 10 %, was injected. Blood samples for measurements of plasma cortisol and plasma ACTH were collected every 15 minutes from 14:00 to 18:00. Before each sampling, 2 ml of blood was drawn and discarded, and after each sampling the catheter was flushed with saline. Ten minutes after sampling, samples were centrifuged at 3000 rpm for 5 minutes at 4 °C, and then stored at –80 °C. A trained bio technician and trained medical students conducted the tests under the supervision of the principal investigator.

3.10.2. Analyses of cortisol and ACTH

Hormones were analysed at the Department of Clinical Biochemistry, Rigshospitalet, Denmark. Plasma cortisol was measured using a competitive electro chemiluminescense immuno assay (ECLIA) (Roche Diagnostica Cortisol) and Modular analytics E170 (Roche). Lower and upper limits of quantitation were 1.0 and 17,500 nmol/l. The interassay coefficients of variation were 4.7 % and 5.6 % at 116 and 968 nmol/l, respectively. Plasma ACTH was measured using a sandwich chemiluminescense immunometric method (ACTH, Immulite Siemens DPC) and Siemens Immulite 2000. Lower and upper limits of quantitation were 1.0 and 556 pmol/l. The interassay coefficients of variation were 7.6 % and 6.1 % at 7 and 106 pmol/l, respectively.

In accordance with Modell et al., cortisol and ACTH responses were calculated according to the trapezoidal rule as the total area under the curve (AUC_{total}) from administration of CRH at 15:00 to the last measure at 18:00.[81] The plasma cortisol (COR) BASAL was estimated as the mean of the

baseline measurements before the administration of CRH and CorPEAK was estimated as the highest plasma cortisol measurement following CRH administration. The primary outcome, the change in plasma cortisol response AUC_{total} (Δ $CorAUC_{total}$), was calculated by subtracting $CorAUC_{total}$ at four weeks from the $CorAUC_{total}$ immediately before the initiation of the intervention. Similarly, Δ was calculated for ACTH AUC_{total}, CorBASAL, and CorPEAK.

3.11. Secondary outcomes

Secondary outcomes included changes in scores from baseline to four weeks on the personality trait neuroticism and cognitive function.

3.11.1. Neuroticism

The personality dimension neuroticism was assessed by the Danish version of the self-rating Eysenck Personality Questionnaire (EPQ),[82;83] and the Revised Neuroticism-Extroversion-Openness-Personality Inventory (NEO-PI-R).[84] The EPQ comprises 101 yes-no items that measure the broad dimensions of neuroticism, extroversion, and psychoticism. NEO-PI-R is a 240-items inventory that evaluates the broad personality dimension of neuroticism, extraversion, openness, agreeableness, and conscientiousness. The score on each of the five broad dimensions is derived by adding the scores from the assessments of six constituent personality traits (facets). The respondent answers the statements on a 5-point Likert scale from 'disagree very much' to 'agree very much'. The outcome measure was the change between the scores for neuroticism on both EPQ and NEO-PI-R applied before (T0) and following four weeks of intervention (T4).

3.11.2. Cognitive function

Cognitive function was measured with a broad battery of neuropsychological tests with relevance to depression, evaluating memory, attention, visuo-motor speed, visuo-constructional abilities, decision making, logical thinking, executive functions and verbal fluency. The following tests were applied: The Danish Adult Reading Test,[85] Familiar faces,[86] Trail Making A and B,[87] Stroop test,[88] Boston naming,[89] Block Designs,[90] Cambridge Cognitive Examination (CAMCOG),[91] Rey Auditory Verbal Learning Test,[92] Rey-Osterrieth Complex Figure,[93] verbal fluency for animals and letter "s",[94] Symbol Digit Modalities Test,[95] and Letter-number sequencing.[96]

All scores of the cognitive tests (except CAMCOG) were transformed to Z-scores with a mean of 0 and an SD of 1 to allow grouping of highly correlated tests into factor scores. Factors scores were computed as the average of constituent test measures and standardized so all factors had a mean of 0 and an SD of 1. Similarly, the averages of all 13 tests measures were computed and standardised to create a global summary, here termed "General Cognition Score". The primary outcome measure of cognitive function was Delta General Cognition Index, calculated as the change in the General Cognition Score from trial entry to after 4 weeks of intervention (T4-T0).

To estimate reliabilities of test measures, we calculated test-retest correlations in all test measures (raw scores, factor scores and General Cognition Score) in the placebo group.

Three students of psychology were trained and supervised by an experienced neuropsychologist and they conducted the neuropsychological testing. All tests were conducted in the same office, and all testing procedures were the same during the study period. The same tester performed both the baseline and the follow up test, which was performed at the same time during the day.

3.12. Assessments

The first part of the assessment was a telephone interview of the potential participants. The individuals eligible were scheduled to meet at the clinic on two different days both before and following four weeks of intervention. On the first day the participants gave written informed consent after details of the trial were explained. Diagnoses were ascertained by the SCAN interview and the structured Clinical Interview for DSM-IV Axis II Personality Disorders.[97] Further assessment included information on family history of psychiatric disorders, ratings of mood using the 17-item Hamilton Depression Rating Scale (HAM-D)[55] and 14-item Hamilton Anxiety Scale,[55] various socio-demographics, height, weight, routine blood tests, and, a pregnancy test for women. Furthermore, following four weeks of intervention blood was drawn for measurements of plasma escitalopram, and the UKU Side Effect Rating Scale[98] was applied by the principal investigator.

3.13. Analysis of plasma escitalopram

The extraction and quantitation of escitalopram was carried out on an ASPEC XL combined with a high-pressure liquid chromatography (HPLC) system, both from Gilson, Villiers le Bell, France. Lower and upper limits of quantitation were 10 and 3,600 nmol/l, respectively. The interassay coefficients of variation ranged from 5.5 % to 8.4% and trueness ranged from 93.2 to 103.0% within the measurement range. Extraction recovery was 38% and carry-over was less than 1%.

3.14. Sample size

The power and sample size estimations were highly hypothetical since the effect of SSRI on the DEX-CRH test in healthy has not been investigated in any prior trials.[73] Thus, the power and sample size calculations were merely guided by a previous case control study in which the difference between healthy with and without a family history of MDD was regarded as possible relevant

difference between the escitalopram and placebo group,[99] reflecting the hypothesis that the increased cortisol response to the DEX-CRH test in individuals with a family history of MDD would decrease as a result of the SSRI intervention to the level of the cortisol response measured in healthy without a family history of MDD. The high-risk study performed by Modell et al.[100] found that healthy high-risk probands of patients with a diagnosis of MDD examined by the DEX-CRH test present with a cortisol AUC-total (mean ± SEM) of 15,064 ± 3,947 nmol x min/l. Further, Modell et al. reported that cortisol AUC-total (mean ± SEM) in healthy individuals with no family history of MDD was 7,773 ± 1,071 nmol x min/l. A clinically relevant effect of escitalopram on the cortisol AUC-total (mean ± SEM) was thus estimated to be the difference in cortisol AUC-total (mean ± SEM) of high-risk probands of patients with the diagnosis of MDD and that of healthy individuals with no family history of MDD. Accordingly, the relevant difference we aimed to detect or reject was 15,064 – 7,773 = 7,291 nmol x min/l. Given a standard deviation (SD) = SEM x $\sqrt{14}$ = 3,947 x 3.7 = 14,768 nmol x min/l provides a power of the trial at a minimum of 60% ($1 - \beta = 0.60$), β being the risk of overlooking a difference in the cortisol AUC-total. Based on these calculations and feasibility due to resources and the availability of first-degree relatives of patients with MDD, we aimed for a full data set of 80 participants.

3.15. Data management

All the data of each participant was kept in a Case Record File, which fulfilled the medical doctors' obligation to keep patient records. In order to maintain blinding, the result of serum escitalopram concentration obtained at end of the intervention, was sent to the CTU that kept it in a locked safety box until the practical part, the initial data analyses of the trial were conducted, and the conclusions drawn. Participants were not registered in The Danish Psychiatric Central Research Register or in any local hospital registers.

3.16. Safety

Procedures for breaking the code for randomization was established for the case of severe adverse reactions, which could have been related to the intervention or if a serious adverse events had occurred. It was the decision of Ulla Knorr and Lars V. Kessing to request emergency breaks, and the CTU could be contacted at any time regarding the practical procedure. The participants could at all times reach Ulla Knorr by mobile phone.

3.17. Statistical methods

The pre-established data analysis plan was published prior to conducting the statistical analyses on data.[73] Data from all randomised participants were analysed, including those with missing data on the DEX-CRH test. Statistical analyses were planned as ANCOVA,[101] but it turned out that the primary outcome measure was not normally distributed, and could not be transformed into a normal distribution. Thus, the outcome in the intervention and the placebo groups were compared by the Mann-Whitney test. Effect sizes were calculated unadjusted and adjusted for design variables, including stratification variables age, sex, HAM-D total score at entry, body mass index at entry, number of daily cigarettes, and concentration of escitalopram in plasma, if the univariate analyses of these variables had a p-value < 0.1.[102] Initially, the drug level measured in each participant was not included in the models as to keep the analysers blinded. Lastly, after every other analysis had been done and conclusions drawn, analyses for the effect of drug-level were performed. Analyses were performed both on complete datasets, as well as datasets on all participants completed by multiple imputation analysis of missing data from the DEX-CRH test (SAS version 9.1).[73]

3.18. Ethical considerations

Information about the trial was presented to potential participants both verbally and in written form in quiet surroundings, and the participants were allowed to bring a relative or friend. It was made clear that participation was voluntary and that the participant could withdraw the given consent at any time without consequence for future treatment possibilities. Participants received a copy of their rights. All participating healthy volunteers signed a written informed consent. The participants were paid up to 9,000 Danish crowns for participation of four to eight days (equal to about one to two weeks pay) and were further compensated for any travel expenses. After the randomisation code was broken the participants received a letter with information on whether they received escitalopram or placebo.

4. Results

4.1. Participants and non-participants characteristics

The probands (n = 466) gave us permission to contact 359 first-degree relatives, who were the potential participants in the trial. The mean age of non-participants was 37 (SD 11) years and 58 % were women. The reasons for their non-participation are presented in Figure 2. The clinical and demographic characteristics of the participants at entry are presented in Table 1.

4.2. Flowchart for the AGENDA trial

A total of 80 participants were included and randomised. The flow chart is presented in Figure 2.

4.3. Adherence to the intervention and adverse events

Two participants randomised to escitalopram were excluded from the trial prior to intervention: one man withdrew his informed consent, and one woman developed skin rash necessitating glucocorticosteroid treatment. No participants left the placebo group, and 33 in

the escitalopram group and 32 in the placebo group stated full adherence to the protocol. Six participants in the escitalopram group and seven in the placebo group stated that they missed taking one or two tablets. A total of 51 % of the participants experienced no side effects, 56 % and 46 % in the placebo and escitalopram group, respectively. No severe adverse reactions or serious adverse events occurred. The side effects assessed by the UKU Side Effect Rating Scale following four weeks of intervention by escitalopram 10 mg (n = 39) or placebo (n = 39) are listed in Table 2. Sexual adverse effects were statistically significantly increased and insomnia was statistically significantly decreased in the escitalopram group compared with the placebo group.

4.4. Plasma escitalopram

Blood was drawn from all 78 participants at follow up, but one test from the escitalopram group failed. The mean concentration of escitalopram was 50 nmol/l, SD 29 nmol/l, median 48 nmol/l, range < 10 to 138 nmol/l, (n = 38). Two participants from the escitalopram group had undetectable plasma escitalopram, thus < 10 nmol/l, one of which had stated missing the last two tablets prior to blood sampling. Plasma escitalopram was undetectable in all participants of the placebo group.

4.5. The success of blinding

The agreement between the actual intervention group and the guess was 'some' demasking (κ = 0.23 (0.01-0.45)) for the participants and 'slight' demasking (κ = 0.18 (0.00-0.40)) for the principal investigator.

4.6. Cortisol and ACTH response in the DEX-CRH test

The two datasets for the DEX-CRH test were complete for 73 participants. As described above, two participants had no tests. Further, one woman and one male missed the baseline test due to schedule

problems. The test following the intervention was missed by two males due to schedule problems and the one male due to technical reasons. The baseline measurements are presented in Table 3. There was no statistically significant difference of the primary outcome Δ $CorAUC_{total}$ comparing the intervention and the placebo groups (Mann-Whitney), (p = 0 .47). In univariate analyses, no statistically significant correlations were found between Δ $CorAUC_{total}$ and the variables: age, sex, HAM-D, body mass index, and number of daily cigarettes, respectively, at randomisation. We found no significant differences between the results of the complete case analysis and the analysis done after multiple imputations. The correlations between plasma escitalopram and Δ $CorAUC_{total}$ were analysed in the escitalopram group. Increasing plasma escitalopram was significantly correlated with decreasing Δ $CorAUC_{total}$ (Friedmanns rho = - 0 .41 (R^2 = 0.046), p = 0.01).

4.6.1. Post-hoc explorative analyses of the DEX-CRH test

The escitalopram group and the placebo group did not separate significantly in analyses of Δ plasma ACTH AUC_{total}, Δ CorBASAL, or ΔCorPEAK, results are presented in Table 4.

In additional analyses we found that the logarithm of AUC_{total} for plasma cortisol before and after the intervention followed a normal distribution with good approximation. Thus, the measure: Δ logCorAUC = $\ln(CorAUC_{total.after}) - \ln(CorAUC_{total.before}) = \ln(CorAUC_{total.after} / CorAUC_{total.before}) = \ln(ratio)$, which has a normal distribution, was analysed. The means of Δ logCorAUC for escitalopram versus placebo did, however, not differ significantly (p = 0.49).

There was a statistically significant interaction for Δ logCorAUC between age and intervention group. Thus, the slope relating to age Δ logCorAUC (p = 0.024) differed significantly between the two intervention groups and the correlations between age and Δ logCorAUC were R^2 = 0.07, Pearson's rho - 0.27, for escitalopram and R^2 = 0.08, Pearsons's rho = 0.28 for placebo.

Data were moreover analysed using mixed model effect analyses (results not presented) and no statistically significant difference between the intervention and the placebo group was found. In

accordance with Modell et al.,[103] a subgroup of 23 individuals with a PEAK cortisol concentration of 110 nmol/l or more in the DEX-CRH test at trial entry was analysed. No statistically significant difference was shown on the Δ $CorAUC_{total}$ for this subgroup ($p = 0.9$). In addition, we analysed the effect of escitalopram on Δ $CorAUC_{total}$ for participants of the escitalopram group that had detectable escitalopram in plasma (n = 36) versus placebo, but no statistically significant difference was found ($p = 0.69$).

4.7. Effects on neuroticism

The dataset was complete with the exception of the one man and the one woman in the escitalopram group who left the trial prior to the intervention, and two men in the placebo group in whom data collection failed for both EPQ, and NEO-PI-R in one, and for only EPQ in another. The baseline data are presented in Table 5.

The change Δ (after minus before) in reported neuroticism scores for participants who took escitalopram compared with placebo participants showed no statistically significant difference, NEO-PI-R ($p = 0.09$) and EPQ ($p = 0.73$), Table 6. No statistically significant correlations were found between changes in neuroticism measured using EPQ or NEO-PI-R, and age, sex, years of education, or plasma escitalopram.

4.7.1. Post-hoc exploratory analyses of personality tests

Post-hoc analyses showed no statistically significant correlations between: Δ EPQ neuroticism and BDI-21 at entry (rho = - 0.26; $p = 0.06$), Δ EPQ neuroticism and HAM-D at entry (rho = 0.12; $p = 0.32$), Δ NEO-PI-R neuroticism and BDI-21 at entry (rho = -0.10; $p = 0.38$), and Δ NEO-PI-R neuroticism and HAM-D at entry (rho = - 0.05; $p = 0.69$). Furthermore, no statistically significant differences were shown in Δ EPQ extraversion ($p = 0.24$), Δ EPQ psychoticism ($p = 0.96$), Δ NEO-

PI-R extraversion ($p = 0.90$), Δ NEO-PI-R openness ($p = 0.33$), and Δ NEO-PI-R conscientiousness ($p = 0.07$) between escitalopram and placebo participants. However, a statistically significant difference was found in Δ NEO-PI-R agreeableness between escitalopram 2.38; 8.09 (mean; SD) and placebo -1.32; 7.94 (mean; SD) ($p = 0.046$), Table 6.

4.8. Cognitive function, (Paper IV)

The dataset for the neuropsychological tests was complete for 77 participants (96 %) both before (T0) and following four weeks of intervention (T4). The test results at entry are presented in the Table 7.

Both groups improved considerably from T0 to T4 in the general cognition score, possibly due to retest effects. The change (Δ) in the general cognitive function score was normally distributed (Shapiro Wilkes test). Accordingly, we tested the difference between the two intervention arms with a t-test, but the difference was insignificant ($p = 0.37$), Table 8. In univariate analyses, no statistically significant correlations were found between the general cognitive function score and age, sex, Hamilton depression score at entry, Danish Adult Reading Test, and plasma escitalopram.

4.8.1. Post-hoc explorative analyses of the results the neuropsychological tests

In post-hoc explorative analyses of the changes of factors 1-4 individually, no statistically significant differences were found between the escitalopram group and the placebo group. For the change in the CAMCOG test, there was a statistically significant difference between the intervention groups, however, contrary to the hypothesis, treatment with escitalopram improved the CAMCOG score less than placebo (1.21 (SD: 1.92) versus 2.16 (SD: 1.98), $p = 0.04$).

5. Discussion

The AGENDA trial is the first trial in which the effect of SSRIs in healthy first-degree relatives of patients with depression has been investigated. In addition, to date the AGENDA trial is the largest trial (n = 80) in which the long-term effect of SSRI is investigated in healthy individuals regardless of outcome.[104] The main finding of this trial was that no statistically significant differences were found between four weeks of intervention with escitalopram 10 mg/day compared with matching placebo on changes in: 1) responses in the HPA-axis, as measured by Δ $CorAUC_{total}$ in the DEX-CRH test, 2) the personality trait neuroticism, and 3) cognitive function, in healthy first-degree relatives of patients with MDD. Thus, our hypotheses that an intervention with escitalopram 10 mg would: 1) decrease the cortisol response in the DEX-CRH test, 2) decrease neuroticism and 3) enhance cognitive function in healthy first-degree relatives of patients with MDD were not supported.

According to the DEX-CRH test no statistically significant effect was found on any other measure of the test, though Post-hoc analyses showed that increasing levels of escitalopram tended to decrease the HPA-response in the DEX-CRH test and this effect increased with age. Thus, activation of the monoaminergic neurotransmitter systems by escitalopram does not seem to substantially affect the HPA-axis as measured by the DEX-CRH test in healthy individuals with a family history of MDD. This finding seems to indicate that intervention with SSRI does not reduce the response to stress in first-degree relatives. Our finding is in accordance with recent data showing that restoration of HPA system dysfunction seems to be neither a necessary nor a sufficient determinant for an acute treatment response in depressed patients.[105] Taken together these findings suggest that dysregulation of the HPA-axis does not play a primary role in the mechanisms of action of SSRIs. The HPA dysregulation seen in depressed patients may rather represent the down stream effects of other, more primary abnormalities as suggested by Manji et al.[106] Further, it is possible

that healthy individuals have modulating homeostatic mechanisms between the serotonergic and the HPA systems that counteract the eventual effect of a SSRI. These changes are possibly not reflected in the response of the DEX-CRH-test.

Regarding personality, no other personality traits with the exception of agreeableness as measured by NEO-PI-R were affected by escitalopram. Results from a recent placebo-controlled trial in patients with major depression suggest that the SSRI paroxetine has a specific effect on the personality traits of neuroticism and extraversion that is distinct from its effect on depression.[107] On the other hand, another study found that reductions in neuroticism correlated with improvement in depression in response to treatment with a SSRI.[108] Our results show that escitalopram has no major direct effect on neuroticism. Regarding our finding of a possible effect of escitalopram on agreeableness, the result ($p < 0.046$) was not significant, when considering the multiple significance testing of the many outcomes of the trial. Furthermore, agreeableness has not been shown to be significantly affected by SSRI treatment (flouoxetine) in a study of depressed patients ($n = 53$).[109]

Regarding cognitive function, no differences were seen between the escitalopram group and the placebo group on any of the neuropsychological tests. The finding in the CAMCOG test is most likely a type 1 error since many outcomes were explored in this trial. Taking multiple testing into account and correcting for that would also make this finding insignificant. In the systematic review of trials investigating the effect of interventions with SSRIs for 7 days or longer,[110] 18 trials that had used 39 different neuropsychological tests to investigate cognitive function in healthy individuals were identified. The findings were inconsistent, thus statistically significant differences[111-117] as well as neutral findings,[111;118-132] were shown. More specifically, in three smaller trials the long-term effect on cognitive function of intervention of escitalopram compared to placebo in healthy

individuals, was investiagted. Two of these studies found no significant effect of escitalopram thus, Wingen et al.[133;134] investigated doses of escitalopram 10-20 mg/day versus placebo for 15 days in a crossover design in 18 participants with an unknown family history of depression. They found no statistically significant effect on actual driving performance, psychomotor performance or visual memory performance. Paul et al.[135] investigated escitalopram 20mg/day versus placebo for 14 days in a crossover design of 24 participants with an unknown family history of depression. They found no effect on psychomotor performance evaluated by multiple tests. In the third and most recent trial, Drueke et al,[136] administered 10 mg of escitalopram for a period of 7 days in a crossover design to 20 healthy male participants with no family history of major mental disorder. They found a differential effect of escitalopram on attention, but found an interaction between serotonin and familiarity with a test of attentional control. Thus, the test results depended on whether the test was applied for the first or the second time in relation to escitalopram and placebo. In this way, the crossover design may induce bias due to the crossover resulting in repeated multiple testing and retest effects on cognitive function. A parallel group design as used in the AGENDA trial may be superior to the crossover design in this context.

5.1. Advantages of the AGENDA trial

This trial has several advantages. First, the trial and the analyses were carried out as planned in advance and the completion in the trial was very high. No participants dropped out due to adverse events. The majority of the participants of the trial experienced no adverse events, however, we observed a statistically significant increase in difficulty with ejaculation (men) in the escitalopram compared with the placebo group. Second, the registered diagnosis of depression for the probands was verified by a face-to-face psychiatric research interview by trained medical doctors at the Department of Psychiatry, Rigshospitalet. The participants in the trial were assessed and diagnosed

by validated and frequently used multi-dimensional methods (SCAN and SCID interviews). Third, the participants were genetically homogeneous as all were ethnic Danes with European, mostly Danish, parents and grandparents. Fourth, we used well established methods, e.g., the DEX-CRH test which is a sensitive, biological, objective test to detect increased HPA function in humans.[137;138] The response to the DEX-CRH test may be sensitive to age[139] and sex,[140] and in our trial, stratification by these variables resulted in equal distributions in the two intervention groups. Fifth, the participants were studied in a randomised clinical trial blinded in all phases including the statistical analyses and conclusion phase. The blinding was successful in relation to participants as well as researchers. Sixth, the antidepressant effect of escitalopram is generally accepted.[141;142] Escitalopram 10 mg was selected because of its specific serotonergic actions.[143] Finally, the participants were subjected to four weeks of intervention thus including the interval in which clinical improvement has been reported in trials with patients with MDD.[144]

5.2. Limitations of the trial

It is a limitation that healthy individuals with a family history of MDD were not compared to healthy individuals without a family history of MDD. We have currently assessed a sample of 40 healthy individuals without a family history of depression and analyses are in progress.

A large number of women were excluded from our trial due to oral contraceptives and pregnancy, thus the trial population is characterized by an overrepresentation of men compared to all first-degree relatives. The exclusion criteria were chosen for safety reasons and to decrease the risk of results being confounded by factors known to substantially affect the HPA-axis, e.g., women taking oral contraceptives, thus interfering with the primary outcome measure.[145]

The selection of the participants happened in many steps from the proband allowing us to contact their adult sibling or child, to the many different motives that the participants expressed for entering the trial like altruism, the opportunity to earn some money, getting to know more about depression, wanting to know the effect of a SSRI on themselves and, wanting to have the experience of being part of a trial. The most frequently expressed reasons not to participate were an unwillingness to take medication that was not indicated by a disease, that participation was to time consuming and that the compensation was too low. Since these reasons for accepting or refusing to participate in the trial point in different directions, we have no reason to believe that the sample selection resulted in an inclusion of very robust "super healthy" or "super unhealthy" participants.

We cannot exclude that the dosage of escitalopram 10 mg/day was too low although this has been suggested as the optimum dose for treatment of moderate depression.[146] Even though, the participants received weekly phone calls to optimise adherence, several of the participants in the escitalopram group were found to have low plasma escitalopram concentrations. We have considered using a higher dosage, but escitalopram 20 mg daily might have given more adverse effects, eventually jeopardizing blinding and adherence, thus it was decided to use 10 mg daily. However, the dose of escitalopram 10 mg resulted in well-known adverse effects. Furthermore, we saw large intra- and inter-individual differences in the DEX-CRH test results, which questions the sensitivity of the test in this sample. Analyses showed that CorPEAK was delayed for some participants when compared to the pattern for depressed patients in the results presented by Modell et al.[147] When designing the trial, we attempted to compensate for this by prolonging the time of terminating the tests from 16.45 to 18:00, but our results suggest that this may still have been too short a period of observation.

5.3. Risk of errors

The risk of errors in trials falls in three major categories.[148;149]

1) *Systematic error ('bias')*: We have minimized bias by using a randomised, age-and sex-stratified comparison with blinding in all phases of the trial. Also our neutral results speak against any bias. 2) *Random error ('play of chance')*: We planned to include 80 participants due to resources and availability of the healthy first-degree relatives of patients with MDD studied in our group. No prior trials have investigated the effect of SSRI on healthy individuals and this made the power calculations were hypothetical and influenced by great uncertainty. In the era of systematic reviews it has been questioned if the size of an individual trial still does matter.[150] The results from any trial may contribute to the larger body of evidence despite arbitrary sample size calculations in the individual trial that may eventually prevent important trials from being conducted.

The AGENDA trial is the first trial including only first-degree relatives of patients with MDD and for this group of individuals the trial may be followed by more trials. Further, our finding may not be a result of decreased statistical power, as the absolute values in the change in the $CorAUC_{total}$ during four weeks of intervention were very low, compared to the large inter-individual values (although these values were higher for the escitalopram group than for the placebo group). Moreover, selecting the more homogeneous subgroup of 23 individuals with high CorPEAK concentration of 110 nmol/l or more in the DEX-CRH test at entry also did not reveal a statistically significant difference between intervention with four weeks of escitalopram and placebo. The AGENDA trial was planned and executed as a superiority trial and was not designed as an equivalence or non-inferiority trial.[151] Hence, we cannot exclude the possibility of overlooking a difference due to the play of chance. Only further trials can solve this issue.

3) *Design errors*: These errors may include that several participants did not reach sufficient levels of escitalopram in the blood in order to produce an effect on the HPA-axis or the other outcomes. The

serum escitalopram concentrations were lower than in the study by Soegaard et al.,[152] who found steady state plasma escitalopram concentrations of 63 ± 32 nmol/l at day 24 for escitalopram 10 mg as compared to 50 ± 29 nmol/l in our trial, though approximately 12 hours elapsed from taking the last tablet to blood sampling in our trial. $CorAUC_{total}$ has previously been suggested to be highly correlated with the serum escitalopram concentration in patients with major depression[153] but in the present trial we found only a weak correlation between drug level and the primary outcome (R^2 = 0.046). Furthermore, we may not have observed the tested participants for an appropriate time period in the DEX-CRH test. Finally, we have analysed multiple outcomes thus increasing the risk of type I error for the secondary and tertiary outcomes of the trial, as previously described.[73]

5.4. Generalizability

Our participants were healthy, ethnic Danes, with a parent or a sibling who was treated for depression in a hospital setting in Denmark but our results, due to neutral findings may generalize to healthy Caucasians in general.

6. Conclusions

The AGENDA trial is the first to investigate the effect of a long-term intervention with escitalopram on serotonin-mediated HPA-axis responses, personality and cognition in healthy first-degree relatives of patients with MDD and the trial is the largest trial that investigated the long-term effect of SSRI in healthy participants on any outcome.

The results did not show a statistically significant difference between escitalopram 10 mg and placebo given for four weeks to healthy first-degree relatives of patients with MDD on predefined primary and secondary outcomes covering the HPA-axis, the personality trait neuroticism and cognitive function.

Post-hoc analyses showed that increasing drug levels of escitalopram tended to decrease the HPA-response in the DEX-CRH test and that this effect increased with age. Further the analyses revealed that treatment with escitalopram compared with placebo might increase the NEO-PI-R personality trait agreeableness.

7. Clinical implications

To infer direct clinical implications from the results were not an aim of our trial, but effects by escitalopram 10 mg on the HPA-axis function, neuroticism and cognitive function was not detected in healthy participants of the trial, thus beneficial effects in healthy may not be abundant.

8. Future studies

Future studies may explore individuals in prodromal phases of depressive disorder, use higher doses of escitalopram or other antidepressants, or establish a run in period to optimize adherence to protocols. Further, future studies may explore the suggested serotonergic link between the personality dimension agreeableness. If the finding of changes in agreeableness is replicated, it may lead to the hypothesis that SSRI do not directly modulate mood but rather mediate a different self-perception captured by changes in the scores of the facets of the personality dimension of agreeableness, which are trust, straightforwardness, altruism, compliance, modesty and tender mindedness.

8.1. Future AGENDA - associations between genepolymorphisms, endophenotypes for depression and antidepressive treatment.

Further analyses of the data collected in the AGENDA trial will be related to the endophenotype paradigm as defined by Gottesman and Guild.[154] Thus, an endophenotype is associated with the

illness in the population, is heritable, is primarily state-independent, co-segregates with illness within families and is found in none-affected family members at a higher rate than in the general population. Recently the term "response endophenotypes" for patients with depression has been suggested.[155] In this paradigm, any early treatment-emergent measures that could be examined within the individual patient could be incorporated. The putative endophenotypes from the AGENDA trial that may show to respond to the intervention by escitalopram may serve as "response endophenotypes" for depression. The analyses of the remaining outcomes of the AGENDA trial may reveal such endophenotypes.[73]

Furthermore, the distinction between healthy with and with out a family history of MDD needs further investigation. Thus, baseline data according to the AGENDA trial has been collected in healthy with no family history of psychiatric disorders, but data has not yet been analysed.

9. Summary

9.1. Summary in Danish / Dansk resume

Virkningsmekanismen for selektive serotonin genoptagelseshæmmere (SSRI) hos patienter med depression er fortsat overvejende ukendt. Der er formentlig en interaktion mellem det serotonerge system og hypothalamus-hypofyse-binyrebarkhormon (HPA) systemet. Yderligere, antages det serotonerge neurotransmitter system at være tæt forbundet til personlighed og kognition. Det er uvist, om SSRI har en direkte effekt på henholdsvis HPA systemet, personlighed og kognition, som er uafhængig af effekten på depression. Muligvis påvirker SSRI disse potentielle biomarkører hos patienter med depression, men det er uklart, om effekten er direkte på de potentielle biomarkører eller om effekten er sekundær til effekten af SSRI på depressive symptomer. Det er ikke tidligere blevet undersøgt, om en intervention med et SSRI har en fordelagtig effekt på disse potentielle biomarkører hos raske med genetisk disposition til depression.

Formålet med studiet er ved et eksperimentelt, medicinsk, blindet, kontrolleret forsøg, at undersøge om langtidsintervention med SSRI versus placebo mindsker cortisolresponset i den kombinerede dexametason corticotropin-releasing hormon (DEX-CRH) test hos raske førstegradsslægtninge til patienter med depression. Yderligere, at teste hypotesen at et SSRI mindsker personlighedstrækket neuroticisme og bedrer kognitiv funktion hos raske førstegradsslægtninge til patienter med depression.

Firs raske førstegradsslægtninge til patienter med depression blev randomiseret til at modtage escitalopram 10 mg eller matchende placebo dagligt i fire uger i et blindet forsøg. Det primære effektmål var interventionsforskellen i ændringen af det totale areal under kurven ($CorAUC_{total}$) for plasmacortisol i DEX-CRH testen ved forsøgets start og efter fire ugers intervention. De sekundære effektmål var ændringen fra forsøgets start til efter fire ugers intervention i: a) selvrapporterede scorer på neuroticismeskalaen i personlighedstestene Revised Neuroticism-Extroversion-Openness-Personality Inventory (NEO-PI-R) og Eysenck Personality Inventory (EPQ), og b) en generel kognitionsscore, som var det standardiserede gennemsnit af 13 testmål for kognitiv funktion. Resultaterne viste, at ændringen i $CorAUC_{total}$ ikke var statistisk signifikant forskellig mellem escitalopram- og placebogruppen, $p = 0.47$. Yderligere påvistes ingen signifikant forskel af escitalopram sammenlignet med placebo på neuroticismescores, NEO-PI-R ($p = 0.09$) og EPQ ($p = 0.73$). Endelig var den gennemsnitlige ændring i den generelle kognitionsscore ikke signifikant forøget i escitalopramgruppen sammenlignet med placebogruppen, ($p = 0.37$). Univariate analyser viste ingen statistisk signifikante korrelationer mellem henholdsvis det primære og de sekundære effektmål i forhold til kovariaterne alder, køn, Hamiltons depressionsscore samt plasmakoncentrationen af escitalopram.

Det konkluderes, at dette forsøg ikke støtter hypoteserne om en effekt af daglig escitalopram 10 mg i fire uger sammenlignet med placebo på HPA-systemet, neuroticisme eller kognitiv funktion hos raske førstegradsslægtninge til patienter med depression.

9.2. Summary in English

The mechanisms of action for selective serotonin re-uptake inhibitors (SSRI) in depressed patients remain widely unknown. The serotonergic neurotransmitter system and the hypothalamic-pituitary-adrenal (HPA) system may interact. Further, the serotonergic neurotransmitter system seems closely linked to personality and cognition. It is not known if SSRIs have a direct effect on the HPA system, personality or cognition that is independent of their effect on depression. Thus, healthy individuals with a genetic liability for depression represent a group of particular interest when investigating if intervention with SSRIs affects these potential biomarkers. SSRIs may affect these potential biomarkers in depressed patients, but it is unclear if the effect is directly on the biomarkers or is secondary to the effect of SSRIs on depressive symptoms. It has newer been tested whether an intervention with a SSRI has a beneficial effect on these potential biomarkers in healthy individuals with a genetic liability for depression.

The aim of the thesis was by an experimental medicine blinded controlled trial, to investigate if long-term intervention with SSRI versus placebo decreases cortisol response in the dexamethasone corticotropin-releasing hormone (DEX-CRH) test in healthy first-degree relatives to patients with major depressive disorder (MDD). Further, to test the hypothesis that a SSRI may reduce neuroticism in healthy first-degree relatives of patients with MDD. Finally, to test whether SSRI enhance cognitive function in healthy first-degree relatives of patients with MDD.

Eighty healthy first-degree relatives to patients with MDD were randomised to receive escitalopram 10 mg versus matching placebo daily for four weeks in a blinded trial. The primary outcome

measure was the intervention difference in the change of the total area under the curve ($CorAUC_{total}$) for plasma cortisol in the DEX-CRH test at entry to after four weeks of intervention. The secondary outcomes were a) change in self-reported neuroticism scores on the 240-items Revised Neuroticism-Extroversion-Openness-Personality Inventory (NEO-PI-R) and the 101-items Eysenck Personality Inventory (EPQ) at entry to after four weeks of intervention and b) the change in the general cognition score, which was the standardised mean of 13 cognitive test measures.

Change in $CorAUC_{total}$ showed no statically significant difference between the escitalopram and the placebo group, $p = 0.47$. Further, escitalopram did not significantly affect self-reported neuroticism compared with placebo, NEO-PI-R ($p = 0.09$) and EPQ ($p = 0.73$). Finally, mean change in the general cognition score was not significantly increased with escitalopram compared with placebo, ($p = 0.37$). In univariate analyses, no statistically significant correlations were found between change in the primary and secondary outcomes, respectively, and the covariates age, sex, Hamilton depression score 17-items, and plasma escitalopram levels.

In conclusion, the present trial does not support an effect of escitalopram 10 mg daily compared with placebo on the HPA-axis, neuroticism and cognitive function in healthy first-degree relatives to patients with MDD.

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Figures and tables

Figure 1. Conductance of the combined DEX-CRH test

Figure 2. Flow chart for the AGENDA trial

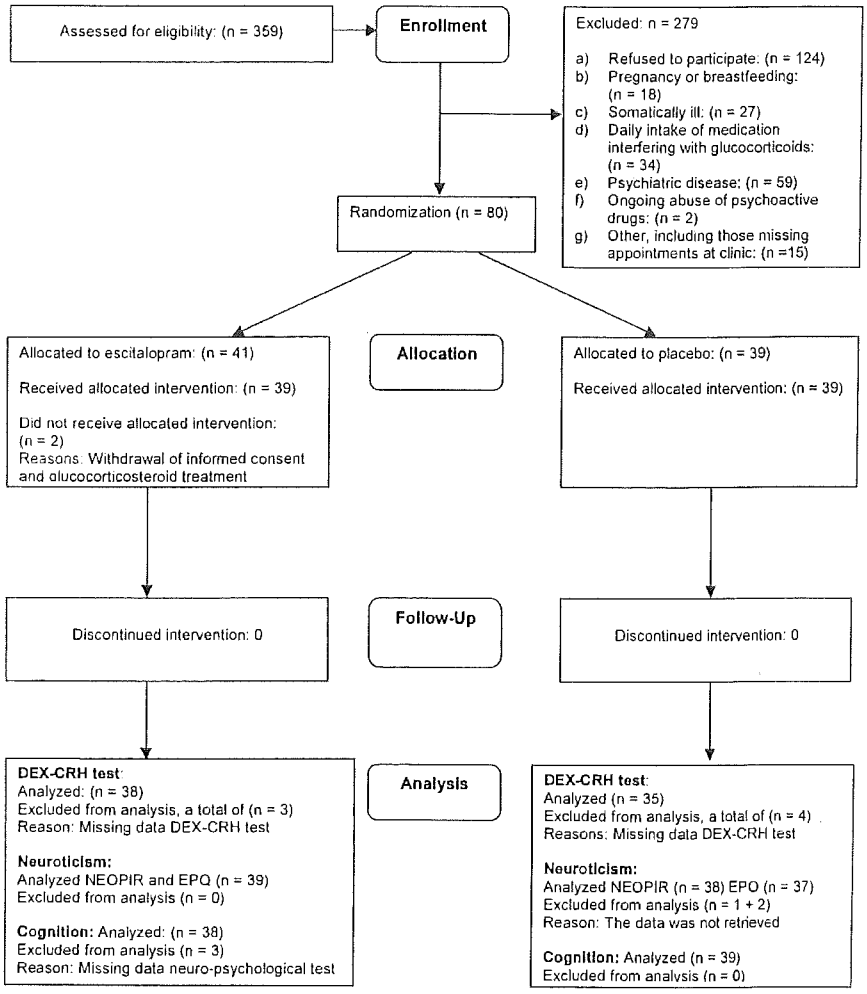

Table 1 – 8

Table 1

The clinical and demographic characteristics of the participants of the AGENDA trial at entry	Escitalopram (N = 41)	Placebo (N = 39)	All Participants (N = 80)
Age – yr, mean ± SD	32 ± 11	31 ± 11	32 ± 10
Women – N (%)	15 (37)	14 (36)	29 (36)
Proband was / – N (%)			
sibling	18 (44)	15 (39)	33 (41)
parent	23 (56)	24 (62)	47 (59)
Caucasian – (%)	100	100	100
Education – mean ± SD			
Years of school	11 ± 1	11 ± 1	11 ± 1
Further education score	3 ± 2	3 ± 2	3 ± 2
Employment status – N (%)			
Employed	30 (73)	26 (67)	56 (70)
Student	11 (27)	11 (28)	22 (28)
Unemployed	0 (0)	2 (5)	2 (3)
Marital status – N (%)			
Single	15 (37)	23 (59)	38 (48)
Married or cohabiting*	26 (63)	16 (41)	42 (52)
First-degree relatives of patient with a history of major depressive disorder – median (quartiles) **	1 (1;2)	1 (1;2)	1 (1;2)
Second degree relatives with a history of major depressive disorder – median (quartiles)	0 (0;1)	0 (0;1)	0 (0;1)
17-item Hamilton Depression Scale Score, – median (quartiles) (range)	1 (0;3) (0-7)	1 (0;3) (0-7)	1 (0;3) (0-7)
14-item Hamilton Anxiety Scale Score, – median (quartiles) (range)	1 (0;2) (0-9)	1 (0;2) (0-6)	1 (0;2) (0-9)
Beck Depression Inventory, 21-item, depression – median (quartiles)	2 (0;4)	2 (0;3)	2 (0;5)
Beck Depression Inventory, 14-item, anxiety – median (quartiles)	1 (0;4)	2 (0;3)	1 (0;3)
Body Mass Index – kg/m^2, mean ± SD	25 ± 4	26 ± 5	26 ± 4
Numbers of daily cigarettes - median (quartiles)	0 (0;11)	0 (0;10)	0 (0;10)
Package years – median (quartiles)	1 (0;10)	2 (0;7)	1.75 (0;8)
Daily medicine – N (%)	2 (5)	4 (10)	6 (8)

Notes: Two smoked cannabis more than two months prior to the investigation. Three were previously abusing alcohol. One participant had generalized anxiety. * Eight were living with their parents. ** quartiles reported, are the 25 and 75 quartiles

Table 2

Side effects assessed using by the UKU rating scale for 78 participants of the AGENDA trial following four weeks of intervention with escitalopram 10 mg or placebo

Side effect	Escitalopram %	Placebo %	p (χ^2)
Restlessness	15	23	0.39
Insomnia	5	23	0.02
Tremor	3	3	1.00
Nausea	10	10	1.00
Diarrhoea	10	3	0.17
Sweating	15	10	0.50
Less libido	18	5	0.08
Erective dysfunction (men)	13	3	0.09
Ejaculating problems (men)	28	3	0.002
Orgasmic dysfunction	28	0	0.000
Headache	3	3	1.00

Table 3

Baseline measurement of the combined DEX-CRH test in the AGENDA trial	Escitalopram (N = 41)	Placebo (N = 39)	All Participants (N = 80)
Plasma cortisol AUC_{total} - nmol/l x min/l, mean ± SD, median (quartiles)*	9045 ± 12829 4691 (2864;8277)	15126 ± 17542 9974 (2549;18336)	12005 ± 15506 5095 (2669;13833)
Plasma ACTH AUC_{total} - pmol/l x min/l, mean ± SD, median (quartiles)**	324 ± 272 255 (209;304)	365 ± 197 306 (233;426)	343 ± 239 263 (215;263)
Plasma cortisol BASAL - nmol/l, mean ± SD, median (quartiles)	15 ± 15 13 (8;17)	24 ± 37 15 (10;20)	19 ± 28 14 (9;18)
Plasma cortisol PEAK - nmol/l, mean ± SD, median (quartiles)	90 ± 124 41 (22;82)	137 ± 153 86 (19;191)	112 ± 140 52 (20;136)

* quartiles reported, are the 25 and 75 quartiles

** There was no statistically significant difference between the escitalopram and the placebo group for any of the hormone measures. AUC_{total} = Area under the curve after administration of CRH corrected for baseline equivalent, BASAL = mean of five measurements at the baseline after pre-treatment with deametasone 1.5 mg and before the administration of CRH, PEAK = the highest measurement following CRH administration.

Table 4

The distributions of the primary ontcome measure and other characteristics of plasma cortisol and plasma ACTH in the combined DEX-CRH test in 73 healthy first-degree relatives of patients with a history of MDD, in the escitalopram 10 mg group (n = 38) and the placebo group (n = 35)

Outcome	Group	Mean (SD)	Median	Minimum value	Maximum value	Interquartile range	p [b]
Δ plasma cortisol AUC_{total} [a]	Escitalopram	1675.1 (13001)	606.6	-40895.6	47913.8	8782.6	0.47
	Placebo	1170.5 (17910)	-200.0	-44680.2	56859.7	7064.2	
Δ plasma ACTH AUC_{total}	Escitalopram	25.1 (158)	-0.08	-392.0	653.0	67.1	0.23
	Placebo	-6.48 (255)	-10.7	-750.0	743.0	108.0	
Δ plasma cortisol BASAL	Escitalopram	0.461 (13.5)	-0.345	-25.4	72.9	4.60	0.57
	Placebo	5.17 (48.4)	0.340	-363	84.1	5.49	
Δ plasma cortisol PEAK	Escitalopram	3.96 (124)	-3.92	-348	356	80.0	0.61
	Placebo	1.76 (131)	1.23	-348	422	69.7	

Δ was the difference between the measurement of plasma cortisol and ACTH after and before four weeks of intervention with escitalopram 10 mg or placebo for:
AUC_{total} = Area under the curve after administration of CRH corrected for baseline equivalent,
BASAL = mean of five measurements at the baseline after pre-treatment with dexametasone 1.5 mg and before the administration of CRH,
PEAK = the highest measurement following CRH administration,
[a] Δ plasma cortisol AUC_{total} was the primary outcome measure
[b] p of Mann Whitney test comparing the two distributions which did not follow normal distributions (Shapiro Wilkes test).

Table 5

Personality traits for 80 first-degree relatives of patients with major depressive disorder in the AGENDA trial			
Personality trait	**Escitalopram (n = 41)**	**Placebo (n = 39)**	**All Participants (n = 80)**
Eysenck			
- mean ± SD, median (25,75 quartiles)			
Neuroticism	6.8 ± 5.3 7 (1.5;10)	7.3 ± 4.4 6.0 (4;10)	7.0 ± 4.8 6.5 (3;10)
Extraversion	16.0 ± 3.8 17 (14.5; 18.5)	14.7 ± 4.5 17 (12;18)	15.4 ± 4.2 17 (14;18)
NEO-PI-R			
- mean ± SD, median (25,75 quartiles)			
Neuroticism	68 ± 24 66 (50;85)	71 ± 18 70 (59-85)	70 ± 21 68 (55;85)
Extraversion	125 ± 19 123 (110;138)	123 ± 16 125 (111;136)	124 ±18 123 (110;136)
Openness	114 ± 17 114 (99;125)	118 ± 18 120 (106;131)	116 ± 74 115 (100;130)
Agreeableness	124 ± 18 125 (118;136)	128 ± 12 128 (119;138)	126 ± 54 127 (118;137)
Conscientiousness	114 ± 20 115 (104;133)	113 ± 17 111 (102;124)	114 ± 18 112 (102;126)

Table 6

Changes in personality scores in the escitalopram and the placebo group following four weeks of treatment in the AGENDA trial.

Personality trait (T4 weeks -T0)	Intervention group	Mean (SD)	Median	Minimum value	Maximum value	Inter quartile range	*p*
Δ Neuroticism[c]	Escitalopram	-1.77 (3.74)	- 1	- 9	12	4	
	Placebo	-2.08 (2.86)	- 2	- 9	4	4	0.73[b]
Δ Neuroticism[d]	Escitalopram	-3.01 (10.3)	- 4	- 31	19	10	
	Placebo	1.00 (10.5)	1	- 21	27	16	0.09[a]
Δ Extraversion[d]	Escitalopram	1.51 (7.95)	2	- 16	18	10	
	Placebo	1.32 (6.24)	2	- 15	15	8	0.90[a]
Δ Openness[d]	Escitalopram	3.18 (9.84)	5	- 30	20	8	
	Placebo	2.15 (9.97)	3	- 17	38	14	0.33[b]
Δ Agreeableness[d]	Escitalopram	2.38 (8.09)	1	- 18	19	11	
	Placebo	-1.32 (7.94)	- 3	- 15	18	11	0.046[a]
Δ Conscientiousness[d]	Escitalopram	1.85 (8.41)	2	- 12	20	14	
	Placebo	-2.34 (11.4)	- 1	- 42	14	14	0.07[a]

a) The distributions did not differ significantly from the normal distribution (Shapiro Wilkes test) and a t-test was used to compare the escitalopram and the placebo arm. b) The distributions differed from the normal distribution but judged from the graphical displays (histograms and probability distributions) they followed normal distributions with reasonable approximation, thus a t-test was also used. c) Eysenck: Escitalopram (n = 39), placebo (n = 37). d) NEO-PI-R: Escitalopram (n = 39), placebo (n = 38).

Table 7

Neuropsychological test results at baseline for 80 first-degree relatives of patients with major depressive disorder whom participated in the AGENDA trial

Neuropsychological test	Mean	Median	SD	25 percentile	75 percentile
Symbol Digit Modalities Test	55	56	9	49	60
Trail Making A	28	27	9	21	35
Trail Making B	63	60	21	49	73
Reys complex figure, 3 min.	22	23	7	19	27
Block designs, seconds	14	12	8	10	16
Fluency for letter s	17	17	5	13	19
Fluency for animals	26	26	6	23	29
Letter number sequencing	12	12	3	11	13
Stroop (incongruence)	107	102	24	91	122
Familiar faces naming	18	20	7	12	24
Boston Naming	56	57	3	53	58
Rey Auditory Verbal Learning Test (A1A5)	50	50	8	43	56
Rey Auditory Verbal Learning Test (delay)	11	10	3	8	13
Cambridge Cognitive Examination (CAMCOG)	97	97	3	96	99

Table 8

The distribution of changes (Δ) in results of neuropsychological test measures in first-degree relatives of the AGENDA trial

Quantity	Arm (n)	Mean (SD)	Median	Minimum value	Maximum value	Inter quartile range	*p*	a) Normality conditions
Δ General Cognition Index	Escitalopram (38)	1.17 (0.552)	1.28	-0.230	2.23	0.89	0.37	N
	Placebo (39)	1.04 (0.693)	1.06	-0.260	2.35	0.97		
Δ Factor 1 Visuo-motor, visuo-spatial function	Escitalopram (38)	0.544 (0.390)	0.488	-0.100	1.55	0.48	0.82	- N
	Placebo (39)	0.423 (0.581)	0.451	-0.640	1.95	0.93		
Δ Factor 2 Executive function	Escitalopram (38)	0.388 (0.581)	0.451	-0.640	1.95	0.93	0.27	N
	Placebo (39)	0.229 (0.639)	0.105	-0.930	1.75	0.84		
Δ Factor 3 Verbal function	Escitalopram (38)	0.255 (0.349)	0.255	-0.340	1.02	0.51	0.86	N
	Placebo (39)	0.239 (0.380)	0.170	-0.590	1.27	0.51		
Δ Factor 4 Verbal learning and memory	Escitalopram (38)	0.952 (0.655)	0.952	-0.610	2.54	0.90	0.41	(N)
	Placebo (39)	1.05 (0.781)	1.16	-0.790	3.38	0.72		
Δ CAMCOG Score	Escitalopram (39)	1.21 (1.92)	1	-5	5	2	0.04	(N)
	Placebo (39)	2.16 (1.98)	2	-2	6	3		

Factor 1: Symbol Digit Modalities Test, Trail Making A and B, Reys complex figure 3 min. and Block designs.
Factor 2: Fluency for letter s, Fluency for animals, Letter number sequencing, Stroop (incongruence).
Factor 3: Familiar faces naming and Boston Naming
Factor 4: Rey Auditory Verbal Learning Test A1A5 and delay.
Δ: The difference (T4-T0) between the measurement after (T4) and before (T0) 4 weeks of intervention with escitalopram 10 mg or placebo.
a) The symbols used in this column are to be interpreted as follows N: the distributions did not differ significantly from the normal distribution (Shapiro Wilkes test), (N) they did differ but judged from the graphical displays (histograms and probability distributions) they followed normal distributions with reasonable approximation. –N: they did not follow normal distributions. In the first case a t-test was applied. In the last 2 cases the distributions were compared using Mann-Whitney test.

Appendix

Trials

BioMed Central

Study protocol

Open Access

Rationale and design of the participant, investigator, observer, and data-analyst-blinded randomized AGENDA trial on associations between gene-polymorphisms, endophenotypes for depression and antidepressive intervention: the effect of escitalopram versus placebo on the combined dexamethasone-corticotrophine releasing hormone test and other potential endophenotypes in healthy first-degree relatives of persons with depression

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Abstract

Background: Endophenotypes are heritable markers, which are more prevalent in patients and their healthy relatives than in the general population. Recent studies point at disturbed regulation of the hypothalamic-pituitary-adrenocortical axis as a possible endophenotype for depression. We hypothesize that potential endophenotypes for depression may be affected by selective serotonin re-uptake inhibitor antidepressants in healthy first-degree relatives of depressed patients. The primary outcome measure is the change in plasma cortisol in the dexamethasone-corticotrophin releasing hormone test from baseline to the end of intervention.

Methods: The AGENDA trial is designed as a participant, investigator, observer, and data-analyst-blinded randomized trial. Participants are 80 healthy first-degree relatives of patients with depression. Participants are randomized to escitalopram 10 mg per day versus placebo for four weeks. Randomization is stratified by gender and age. The primary outcome measure is the change in plasma cortisol in the dexamethasone-corticotrophin releasing hormone test at entry before

intervention to after four weeks of intervention. With the inclusion of 80 participants, a 60% power is obtained to detect a clinically relevant difference in the primary outcome between the intervention and the placebo group. Secondary outcome measures are changes from baseline to four weeks in scores of: 1) cognition and 2) neuroticism. Tertiary outcomes measures are changes from baseline to four weeks in scores of: 1) depression and anxiety symptoms; 2) subjective evaluations of depressive symptoms, perceived stress, quality of life, aggression, sleep, and pain; and 3) salivary cortisol at eight different timepoints during an ordinary day. Assessments are undertaken by assessors blinded to the randomization group.

Trial registration: Local Ethics Committee: H-KF 307413

Danish Medicines Agency: 2612-3162.

EudraCT: 2006-001750-28.

Danish Data Agency: 2006-41-6737.

ClinicalTrials.gov: NCT 00386841

Background

Robins and Guze described five phases in the development of a valid classification of psychiatric illness: clinical description, laboratory studies, delimitation from other disorders, follow-up studies and family studies [1]. Later, response to treatment was added as a sixth phase [2]. Recently, the endophenotype concept has emerged as a strategic tool in neuropsychiatric research [3].

Endophenotypes are quantifiable components in the "genes-to-behaviours" pathways distinct from psychiatric symptoms [3]. In parallel with the classification of psychiatric diseases, endophenotypes are validated by specificity, state independence, heritability, familial association, co-segregation, and biological and clinically plausibility [4].

Several possible endophenotypes have been proposed in affective disorders, including stress regulation, cognition, neuroticism, depression and anxiety symptoms [4]. Pharmacological anti-depressants may have an effect on endophenotypes in healthy persons with a family history of depression. We hypothesized that treatment response could be added to the validation of possible endophenotypes for depression. However, a systematic search for randomized multiple-dose, placebo-controlled trials on the effect of selective serotonin reuptake inhibitors did not identify any trials in which healthy first-degree relatives of depressed patients received a selective serotonin re-uptake inhibitor for at least one week (unpublished data).

Possible endophenotypes for depression

Hypothalamus-pituitary-adreno-cortical (HPA) axis regulation

Impaired regulation of the HPA axis during an acute episode of depression is the most consistent laboratory finding [5-7]. The combined dexamethasone (DEX)-corticotrophine releasing hormone (CRH) test is a sensitive test for detecting altered HPA axis regulation [8]. In this test, the stimulating effects of 100 μg CRH on corticotrophin (ACTH) and cortisol are examined under the suppressive action of 1.5 mg of dexamethasone. ACTH and cortisol in the combined DEX-CRH test demonstrated an exaggerated response in patients with depression compared to healthy controls with a family history of depression [9] and between healthy first-degree relatives of patients with depression compared to healthy controls without a family history of depression [9]. Increased ACTH and cortisol in the combined DEX-CRH test therefore seems to be a promising biomarker for depression and a potential endophenotype for depression [10].

Cognition

Alterations in cognitive functions are common and included in the diagnostic criteria for depression [11] and some patients experience cognitive dysfunction even in euthymic phases of the disease [12-16]. A high-risk study showed impairment of selective and sustained attention, executive function, language processing and working and declarative memory in subjects with a family history of depression as compared to participants without [17]. Thus cognitive function may be a candidate endophenotype for affective disorders.

Personality

Neuroticism is a measure of an individual's tendency to experience negative emotions that are manifested at one extreme as anxiety, depression, and moodiness, and at another extreme, as emotional stability. Neuroticism is most frequently measured by questionnaires such as Eysenck Personality Questionnaire (EPQ) [18] and the revised NEO Personality Inventory (NEO-PI-R) [19]. The heritability of neuroticism is well established [20]. Studies

of healthy first-degree relatives of patients with a depression point at neuroticism as an endophenotype for depression [21].

Other potential endophenotypes for depression

More than 90% of depressed patients complain about impairment of sleep quality, which has been suggested as a potential endophenotype for depression [22]. The effect of antidepressants on sleep quality in healthy individuals with a family history of depression has not been investigated. Subjective measures of stress, aggression, pain, and quality of life are all factors known to improve with remission of depressive symptoms. Whether this is a direct effect of treatment with antidepressants or a consequence of improvement in depressive symptoms is unclear.

Proportion of possible endophenotypes for depression

Based on results from recent studies it is estimated that 30% of healthy persons with a family history of depression will exhibit at least two of the three possible endophenotypes: dysregulation of the HPA axis, cognition, and neuroticism [23]. The prevalence of other possible endophenotypes is unknown.

The effect of antidepressants on endophenotypes for depression

Treatment with antidepressants in patients with an acute depression is associated with partial normalization of the HPA axis [24,25], enhanced cognitive function [26], and reduction in the personality trait of neuroticism [27]. In these trials it has not been possible to distinguish the treatment effect on the endophenotypes from the treatment effect on the disease, since remission of depressive symptoms is associated with partial normalization of the endophenotypes. It is not known whether the treatment responses on the disease symptoms are mediated through an effect on the endophenotypes or vice versa. Results of recent randomized, placebo-controlled trials suggest that antidepressants have an effect on psychological variables and behaviour in individuals without psychiatric illness [28-30], one of these studies did not mention the family history status of the included individuals [28] but in two of the studies [29,30] healthy individuals with a family history of psychiatric illness were excluded.

In summary, no trial has investigated the effects of antidepressants on possible endophenotypes in healthy individuals with a family history of depression.

Genotyping

A recent systematic review and meta-analysis of pharmacogenetic studies of antidepressant response suggests that polymorphisms in genes such as 5-HTTLPR, STin2, HTR1A, HTR2A, TPH1, and BDNF may modulate antidepressant response [31], but the association between gene polymorphisms and the effect of an antidepressant treatment on the putative endophenotypes for depression has not been explored.

Objectives

With the AGENDA trial (Associations between Gene-polymorphisms, Endophenotypes for Depression and Antidepressive Intervention) we want to test the hypothesis that potential endophenotypes for depression are affected hy intervention with an antidepressant in healthy first-degree relatives of patients with the diagnosis of depression.

Methods

The AGENDA trial is designed as a participant, investigator, observer, and data-analyst-blinded randomized trial in which participants receive either escitalopram 10 mg or placeho for a period of four weeks (Figure 1).

Study organization

The study is conducted at the Department of Psychiatry, Rigshospitalet, Copenhagen University Hospital, Denmark as part of the Centre for Pharmacogenomics, University of Copenhagen. The trial has a data monitoring and safety committee (DMSC) that is independent of the investigators conducting the trial.

Participants

Participants are recruited as healthy first-degree relatives of patients with a diagnosis of depression given at discharge from psychiatric hospital in- or out-patient contact [32]. These patients participated in ongoing studies at the psychiatric department of Rigshospitalet, University of Copenhagen, Denmark. Individuals meeting the inclusion criteria and none of the exclusion criteria are enrolled in the trial (Appendix 1). The exclusion criteria were chosen partly for safety reasons and partly to decrease the risk of results being confounded by factors known to substantially affect the HPA-axis, thus interfering the primary outcome measure. Women taking birth control pills were instructed to discontinue these 6 weeks prior to entering the trial. All women were carefully instructed to use double barrier birth control methods and pregnancy tests were performed both before and after the intervention.

Interventions

The participants are randomized to receive either escitalopram or placebo by oral administration as a single dose of 10 mg each evening as self-medication at home for four weeks. On completion of four weeks of double-blind intervention (or early discontinuation from the trial) participants entered a five-day blinded down-titration period to nil medication. Escitalopram 10 mg was selected because of its specific serotonergic selectivity [33] and because of the favourable adverse reaction profile [34], thus facilitating blinding. The dose of escitalopram 10 mg for four weeks was estimated to have a sufficient effect,

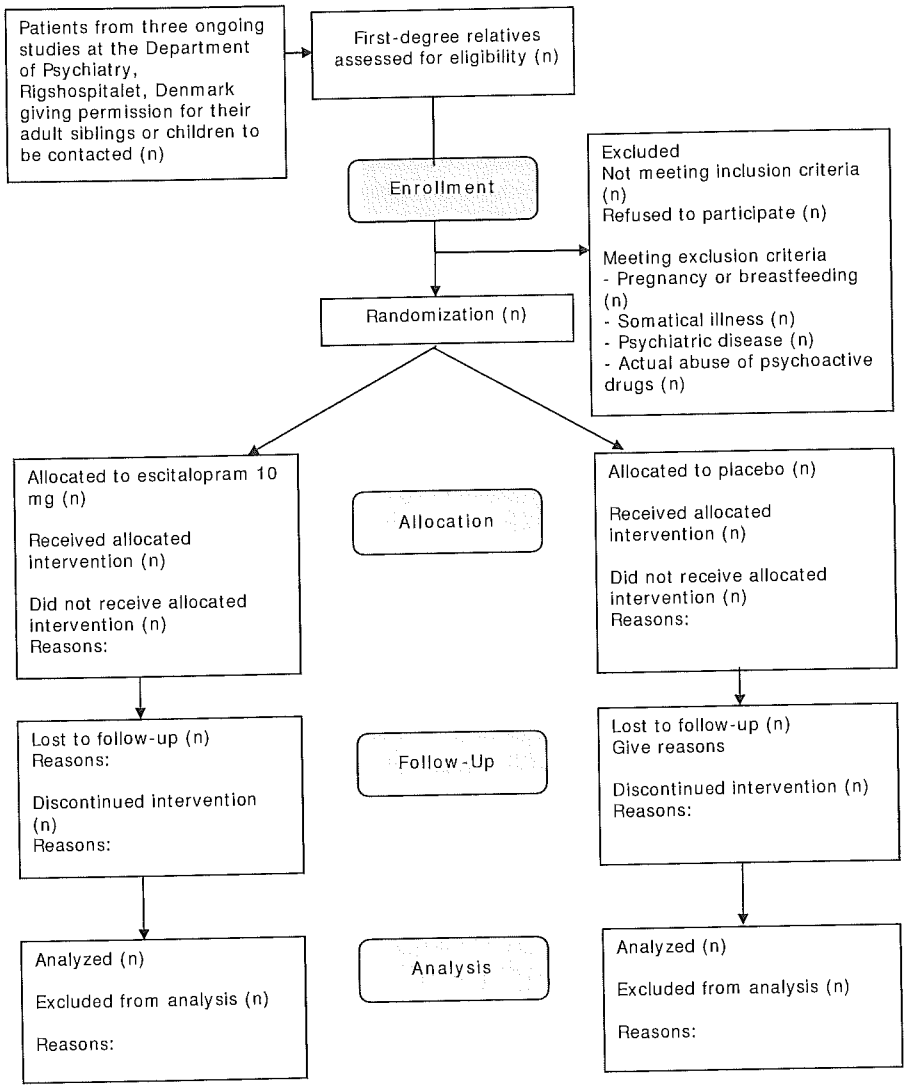

Figure 1
Flowchart for The Agenda Trial.

since effect on depressive symptoms in patients with a major depression is observed within one to four weeks as compared with placebo [34]. The validity of the results depends on a high compliance and high completion in the trial. This was sought obtained by weekly telephone control calls to the enrolled participants to insure adherence to the protocol and to record adverse events. Escitalopram and placebo tablets were identical in appearance, colour, smell, and solubility allowing for blinding of treatment assignment. H. Lundbeck A/S provided identically appearing blister packages containing escitalopram or placebo. An independent pharmacist then packed, sealed and numbered the drug packages according to a randomization list provided and concealed by the Copenhagen Trial Unit (CTU), Centre for Clinical Intervention Research, Rigshospitalet.

Randomization

Randomization into one of the two intervention groups is done immediately after it has been established that the participant fulfils all the inclusion criteria and none of the exclusion criteria. Randomization is stratified by age (18 – 31 years and 32 – 60 years) and sex in order to get an equal distribution in the intervention groups, knowing that the response to the DEX-CRH test is sensitive to these factors. Participants are randomized in a 1-to-1 ratio to receive escitalopram 10 mg or placebo. The Copenhagen Trial Unit (CTU), Centre for Clinical Intervention Research, Rigshospitalet performs the centralised randomization, and only the IT Manager of the CTU will know the block size used for stratification. The sponsor-investigator (UK) provides information of the participants to the CTU during the entry assessment as soon as participation in the study has been decided. CTU performs the computer-generated randomization to ensure adequate allocation concealment and adequate generation of the allocation sequence [35]. The number of the allocated treatment is communicated to UK, both by phone and email.

Blinding

All study personnel and participants are blinded to the packaging of the study drug, and blinding is maintained throughout monitoring, follow-up, data management, assessment of outcomes and data analyses. The randomization code will not be broken until all the data has been analysed and conclusions drawn. At the assessment after four weeks intervention, every participant, the sponsor-investigator (UK), and the neuropsychological testers will make a guess as to which intervention the participant has received. When the trial is finished, inter-rater reliability between the actual intervention and the guesses will be estimated to assess the degree to which blinding has been successful.

Outcome assessments

Outcomes are assessed at entry and after four weeks intervention by assessors who are all blinded to the randomization group.

Depression has a wide range of possible features that can be measured and tested as possible endophenotypes [36]. Since no prior trial has investigated the effect of SSRI on healthy first-degree relatives of patients with depression, we have chosen to include many outcomes since the nature of this trial is predominantly exploratory, i.e., hypothesis generating, and only partly hypothesis testing. When available, previously developed and validated scales and instruments were used in order to facilitate appropriate comparisons with data obtained in previous studies.

Primary outcome

During selection of the primary outcome, it was stressed that the outcome should be objective and of clinical importance. Further, blinding should be possible at all levels of assessment and analyses. The measurement of change in plasma cortisol in the DEX-CRH test from entry before to four weeks during intervention fulfils these criteria. Plasma cortisol will be estimated as the total area under the curve (AUC-total) from administration of CRH at 15.00 hours to the last plasma cortisol measure at 18.00 hours. The DEX-CRH test is performed according to international standards with a few minor modifications [9]; thus plasma cortisol, plasma ACTH and salivary cortisol are measured at the same time points.

Secondary outcomes

Secondary outcomes include changes in scores from baseline to four weeks on: 1) cognitive functions are measured with a broad battery of neuropsychological tests with relevance in relation to depression, evaluating memory, attention, visuomotor speed, visuo-constructional abilities, decision making, logical thinking, executive functions, verbal fluency, social and moral cognition, recognition of emotions and emotional intelligence [37-54], 2) neuroticism as measured by the NEO-PI-R [19] and EPQ [18].

Tertiary outcomes

Tertiary outcomes include changes in scores from baseline to four weeks on: 1) mood as measured by the Hamilton rating scale for depression (HAM-D17) [55] and the Hamilton rating scale for anxiety (HAM-A14 [56], 2) participants subjective perception of pain on a visual analogue scale modified from Klepstad [57], 3) sleep on a visual analogue scale for sleep quality, sleep items from the HAMD-17 and supplementary questions on sleep characteristics, 4) aggression by The Buss-Perry Aggression Ques-

tionnaire [58], 5) depressive symptoms hy the Beck Depression Inventory (BDI-21) [59], 6) quality of life by the WHO Quality of Life questionnaire [60], 7) a global measure of stress by the Cohen Perceived Stress Scale [61], and 8) salivary cortisol assessed by Salivettes during an ordinary day in the participants usual environment at the following time points: awakening, awakening + 15 min., awakening + 30 min., awakening + 45 min., awakening + 60 min., 12:00, 18:00 and at 23:00 [62]. Further, side-effects are assessed at four weeks by the UKU Side Effect Rating Scale [63].

Genotyping

Blood samples are stored in a bio bank for further analyses. Among others we intend to genotype for the 5-HTTLPR-sbort/long-promoter variant, and the catechol-O-methyltransferase (COMT), and to test whether these genotypes will be associated with the response to escitalopram on the potential endophenotypes for depression. Further, messenger RNA for the glucocorticoid receptor will be analysed and associated to the effect of escitalopram.

Assessments

Participants are subjected to almost identical sequences of assessments at entry and after four weeks of intervention (Appendix 2). The first part of the assessment is a telephone interview, and the individuals who are not excluded at that time point, are scheduled to meet at the clinic at two different days before and following four weeks of intervention with escitalopram or placebo. At the first day of examination the participants are interviewed to evaluate fulfilment of inclusion and exclusion criteria including the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [64] and various socio-demographics.

Sample size

A high-risk study performed by Modell et al. [9] found that healthy high-risk probands of patients with a diagnosis of depression examined by the DEX-CRH test present with a cortisol AUC-total (mean ± SEM) of 15,064 ± 3,947 nmol × min/L. Further, Modell et al reported that cortisol AUC-total (mean ± SEM) in healthy individuals with no family history of depression was 7,773 ± 1,071 nmol × min/L. A clinically relevant effect of escitalopram on the cortisol AUC-total (mean ± SEM) was thus estimated to be tbe difference in cortisol AUC-total (mean ± SEM) of high-risk probands of patients with the diagnosis of depression and that of healthy individuals with no family history of depression. Accordingly, the relevant difference we aim to detect or reject is 15,064 – 7,773 = 7,291 nmol × min/L. Given a standard deviation (SD) = SEM × $\sqrt{14}$ = 3,947 × 3.7 = 14,768 nmol × min/L provides a power of the trial at a minimum of 60% (1 - β = 0.60), β being the risk of overlooking a difference in the cortisol AUC-total. However, the power in the trial may be higher, considering the use of analysis of covariance (ANCOVA) of the change from entry to after four weeks of intervention in AUC total during the DEX-CRH test. Based on these calculations we aim for a full data set of 80 participants to be able to conclude in relation to the primary outcome measure.

Statistical methods

All data analyses will be carried out according to a pre-established analysis plan. The main null-hypothesis to he tested is that there is no difference between the two intervention arms with regard to the plasma cortisol AUC-total in the DEX-CRH test. All randomized participants will be analysed, including those with missing data on AUC.

Statistical analyses will be performed using ANCOVA [65]. Thus, the outcomes will be analysed as the difference for the individual participant's before and after the intervention, firstly unadjusted and then adjusted for a number of variables, if they present with a p-value < 0.1 in the univariate analyses [66], see Table 1. Initially the drug level measured in each participant will not be included in the models in order as to keep the analysers blinded. Later on analyses for the effect of drug-level will be performed along with other significant covariates in the multivariate model. Separate analyses will be performed in a log linear model of the changes in the primary outcome as compared to the secondary and tertiary outcomes.

In the case of missing data according to the primary outcome, analyses will be performed both on complete data sets, as well as data sets on all participants completed hy multiple imputation (MI) of missing data by MI-analysis (SASS version 9.1 or NORM version 1) based on age, sex, body mass index, HAMD, neuroticism before and after the intervention, years of education, and AUC-total for cortisol, and ACTH and salivary cortisol hefore and after the intervention. In the case of discrepancy between these results, the result from the MI procedure is regarded as the result in the trial. $P < 0.05$ will be regarded as statistically significant.

Data management

All the data of each participant is kept in a Case Record File, which fulfils the Danish law for medical doctors' obligation to keep patient records. In order to maintain blinding, the result of serum escitalopram concentration at end of the intervention is sent to the CTU, that keeps it in a locked safety box until the practical part and the data analyses of the trial are finished. Participants are not registered in The Danish Psychiatric Central Research Register or in any local hospital registers.

Table 1: Covariates in the statistical models for the AGENDA trial.

Outcome	Primary outcome: AUC-total plasma cortisol	Secondary outcome Neuroticism	Secondary outcome Cognitive function	Tertiary outcomes
AUC-total plasma cortisol	x	x		x
Age	x	x	x	x
Gender	x	x	x	x
HAMD, entry	x		x	x
Body Mass Index, entry	x			
Number of daily cigarettes	x			
Danish Adult Reading Test			x	
Years of education		x		
Drug-level	x	x	x	x

Safety

Procedures for breaking the code for randomization has been established for the case of severe adverse reactions, which can be related to the intervention or if a serious adverse events occur. It is the decision of UK and LK to request emergency breaks, and the CTU can be contacted at any time regarding the practical procedure. The participants can at all times reach UK by mobile phone. An independent data monitoring and safety committee has been established to further ensure the safety of the participants, should the need for considering early stopping occur.

Results

Current trial status

Enrolment started July 2007 and is ongoing until July 2009. Status in May 2009 is that 390 eligible persons have been screened and that 77 of these have been randomized (Figure 1) and the dataset is complete for 64 participants regarding the primary outcome measure.

Ethical considerations

The regional ethics committee for the greater Copenhagen area has approved the protocol (H-KF-307413) as has The Danish Data Protection Agency (2006-41-6737) and The Danish Medicines Agency (2612-3162). The trial has the EudraCT number 2006-001750-28 and is registered at ClinicalTrials.gov as NCT 00386841. Both positive, neutral, and negative findings from the trial will be published in accordance with the CONSORT guidelines [67]. The trial is conducted and monitored in accordance with the International Conference on Harmonization for Good Clinical Practice guidelines [66] and the Declaration of Helsinki 2002 http://www.wma.net/e/policy/b3.htm.

Information about the trial is presented to potential participants both verbally and in written form in quiet surroundings, and the participants were given permission to bring a relative or friend. It is made clear that participation is voluntary and that the participant can withdraw the given consent at any time without consequence for future treatment possibilities. Participants receive a copy of their rights. All participating healthy volunteers sign a written informed consent. The participants are paid up to 9,000 Danish crowns for full participation (equal to about one weeks pay) and are further compensated for any travel expenses. When the randomization code is broken the participants will receive a letter with information on whether they received escitalopram or placebo and the major results of the trial.

Discussion

The AGENDA trial is the first trial investigating whether an antidepressant has an effect on potential endophenotypes in healthy first-degree relatives of patients with a diagnosis of depression. This represents a new strategy to validate potential endophenotypes for depression, which may cast light on the pathophysiology of depression. The trial is fully investigator initiated and controlled to secure unbiased assessment of the effect of escitalopram on endophenotypes of healthy first-degree relatives of patients with depression. The AGENDA trial has received non-restricted grants from non-profit and for-profit organizations.

Perspectives

Further knowledge of endophenotypes may increase the validity of the diagnosis of depression in the future and may eventually improve our possibilities to reclassify depression. The principle of testing the effect of a psychotropic drug on possible endophenotypes for a given disorder could be used to test putative endophenotypes for other disorders as well.

Further outcome assessments

We plan to assess the effect of escitalopram versus placebo on a number of other outcomes in healthy participants with a family history of depression. These assessments include:

A: Investigation of the association between inflammation and depression. A flow-cytometric profile with focus on activated and non-activated t-cell subsets measured before and after intervention. Furthermore inflammatory variables and proinflammatory genepolymorphisms are measured.

B. Magnetic Resonance Imaging (MRI) of hippocampus volume. Studies of patients with unipolar depression suggest a decreased volume of hippocampus in MR scans [68]. Decreased volume of hippocampus is a possible endophenotype for depression and the effect of SSRI on hippocampal volume has not been established in healthy individuals with a family history of depression. Functional MRI including Face Emotion – Gender Discrimination Task [69] and Flanker Go-No go Risk paradigm [70] is conducted in a subpopulation of participants in the AGENDA trial.

Competing interests

UK, MK, UFR, LH, AG, EH, OP, JW, and CG declare to have no competing interests.

MV has been a speaker for Eli Lilly, Wyeth, AstraZeneca and Pfizer, and is a member of the advisory board for AstraZeneca A/S, Denmark. LK has been a consultant for Bristol-Myers Squibb, Eli Lilly, Lundbeck, AstraZeneca, Pfizer, Wyeth, and Servier.

Authors' contributions

All authors were involved in the conception and design of the study protocol, drafting or revising the manuscript, and have approved the final manuscript. UK and LK are the clinical investigators. UK is the sponsor and co-ordinating investigator and is responsible for inclusion of participants and research interviews. UK, LK, JW, and CG are responsible for the data analysis. All authors will participate in interpretation of results and in the writing of subsequent papers.

Appendices

Appendix 1. Criteria for inclusion and exclusion in the AGENDA trial

Inclusion criteria

- Healthy individual of both sexes. Women should preferable be in day 1–13 of her menstrual cycle at the time of randomization.
- Offspring or sibling of an ethnic Dane, with a history of psychiatric in- or outpatient care with the diagnosis of depression and who later had the diagnosis verified in a SCAN interview at the Department of Psychiatry Rigshospitalet, Denmark 2004–2009.
- Aged 18 – 60 years.
- Born in Denmark.
- European parents and grandparents.
- Able and willing to sign informed consent.

Exclusion criteria

- Somatical illness or other handicap, which make participation in the trial impossible.
- Daily intake of drugs interfering with corticosteroids or escitalopram, including birth control pills or any kind of corticosteroids.
- Hypersensitivity to escitalopram, dexamethasone, or human corticotrophin-releasing hormone.
- Former medical or psychological treatment for diseases in the affective or schizophrenic spectrum.
- Abuse of alcohol or psychotropic medication.
- For women: pregnancy or breastfeeding.

Appendix 2. Assessments of prognostic factors and outcome measures in the AGENDA trial

Basic information

Socio-demographics, family history of psychiatric illness, Kendler's questionnaire for lifetime events in a brief Danish version [71], Schedules for Clinical Assessment in Neuropsychiatry, version 2.1[64], The Structured Clinical Interview for DSM – IV Axis II Personality Disorders [72], birth weight, height, current weight, waist-hip ratio and

Edinburgh Inventory for handedness [73]. For women a pregnancy test is performed.

At entry and at four weeks of intervention the following variables are assessed

HPA-Axis

The Combined Dexamethasone Corticotrophine-releasing Hormone Test (DEX-CRH) salivary cortisol, plasma cortisol and plasma-ACTH are measured every 15 minutes from 14:00 – 18:00 hours [9].

Cognition

The Danish Adult Reading Test [37], Familiar faces [38], Trail Making A and B [39], Stroop test [74], Boston naming [40], Block Designs [41], Cambridge Cognitive Examination [43], Rey Auditory Verbal Learning Test [44], Rey-Osterrieth Complex Figure [45], verbal fluency for animals and letter "s" [46], Symbol Digit Modalities Test [47], Iowa Gambling Task [48], Letter-number sequencing [49], recognition of facial emotions [54], moral judgement [51,52], Mayer-Salovey-Caruso Emotional Intelligence Test [53].

Personality

Eysenck Personality Questionnaire [18] and NEO Personality Inventory revised, computer version [19].

Roting scales of mood

Hamilton Depression Scale 17-items [55] and Hamilton Anxiety Scale, 14-items [56].

Depressive symptoms, quality of life, perceived stress, subjective evaluations of aggression, sleep and pain

Buss-Perry Aggression Questionnaire [58], Beck Depression Inventory, 42-items [59], Side Effect Self Rating Scale by UKU-SERS-Pat [63], WHO Quality of Life [75], Cohen's Perceived Stress Scale [61] and Klepstad Visual Analogue Scale for pain, modified [57]. Sleep is evaluated by Hamilton Depression Scale and additional questions of number of night sleep hours, subjective quality of sleep on a visual analogue scale and number of interruptions of sleep.

Salivary cortisol

Saliva samples by Salivettes for measurements of cortisol are obtained at the clinic and during a day in the participant's usual environment.

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Escitalopram and neuroendocrine response in healthy first-degree relatives to depressed patients – a randomized placebo-controlled trial

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ABSTRACT

Background: The mechanisms by which selective serotonin re-uptake inhibitors (SSRI) act in depressed patients remain unknown. The serotonergic neurotransmitter system and the hypothalamic-pituitary-adrenal (HPA) system may interact. The aim of the AGENDA trial was to investigate whether long-term intervention with SSRI versus placebo affects the cortisol response in the dexamethasone corticotropin-releasing hormone (DEX-CRH) test in healthy first-degree relatives to patients with major depressive disorder (MDD).

Methods: Eighty healthy first-degree relatives to patients with MDD were randomized to escitalopram 10 mg versus matching placebo daily for four weeks. The primary outcome measure was the intervention difference in the change of the total area under the curve ($CorAUC_{total}$) for plasma cortisol in the DEX-CRH test at entry to after four weeks of intervention.

Results: Change in $CorAUC_{total}$ showed no statically significant difference between the escitalopram and the placebo group, $p = .47$. There were large intra- and inter-individual differences in the results of the DEX-CRH test. There was statistically significant negative correlation between the plasma escitalopram concentration and change in $CorAUC_{total}$, rho = - 0.41, $p = .01$. Post-hoc analyses showed a statistically significant interaction between age and intervention group and change in log $CorAUC_{total}$.

Conclusion: The present trial does not support an effect of escitalopram 10 mg daily compared with placebo on the HPA-axis in healthy first-degree relatives to patients with MDD. Increasing levels of escitalopram tended to decrease the HPA-response in the DEX-CRH test and this effect increased with age.

Trial registration

ClinicalTrials.gov: NCT 00386841, (AGENDA).

Local Ethics Committee: H-KF 307413.

Danish Medicines Agency: 2612-3162.

EudraCT: 2006-001750-28.

Danish Data Agency: 2006-41-6737.

Key words

escitalopram; healthy; trial; randomized; hypothalamic-pituitary-adrenal axis; combined dexamethasone corticotropin releasing hormone test; major depressive disorder

Introduction

Depression is associated with an altered function of the hypothalamic-pituitary-adrenal (HPA) axis, including increased cortisol responses to the dexamethasone corticotropin releasing hormone (DEX-CRH) test (17). Previous studies have shown that even healthy first-degree relatives to patients with major depressive disorder (MDD) have an abnormal HPA response to the DEX-CRH test, with an intermediary response when compared to healthy controls and patients with major depression (25). Furthermore, salivary cortisol has been shown to be increased in individuals with a family history of MDD as compared to healthy individuals without a family history of MDD (14;24;32). Intervention with a single dose of a selective serotonin re-uptake inhibitor (SSRI) has been found to increase serum corticosterone levels in rats (9;18) and plasma corticosteroid levels in healthy humans (7;23;26;27;29).

It has newer been investigated whether this deregulated HPA axis in healthy individuals with a family history of MDD may become normalized by antidepressants (19). Thus, the AGENDA (**A**ssociations between **g**enepolymorphisms, **e**ndophenotypes for **d**epression and **a**ntidepressant intervention) trial (20) is the first to investigate the effect of long-term (four weeks) daily administration of a selective serotonin re-uptake inhibitor (SSRI) versus placebo on the HPA-axis in healthy first-degree relatives to patients with MDD (19). The function of the HPA-axis was investigated using the DEX-CRH test. The aim of the present trial was to test the hypothesis that an intervention with SSRI as compared with placebo affects the cortisol response in the DEX-CRH test for first-degree relatives of patients with MDD.

Methods and Materials

The AGENDA trial was investigator initiated and designed. It was conducted as a participant-, investigator-, observer-, and data-analyst-blinded trial. During the trial the participants received

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either escitalopram 10 mg/day or placebo for a period of four weeks. The trial protocol was published ahead of trial completion (20). The trial was conducted from July 2007 until July 2009 at the Department of Psychiatry, Rigshospitalet, Denmark as part of the Centre for Pharmacogenomics, University of Copenhagen, Denmark. The trial was conducted and monitored in accordance with the International Conference on Harmonization for Good Clinical Practice guidelines (3) and the Declaration of Helsinki 2002 (www.wma.net/e/policy/b3.htm).

Probands

Probands were patients with MDD from psychiatric in- or out-patient hospital contact in Denmark who participated in ongoing studies at the Department of Psychiatry, Rigshospitalet. Their diagnoses were validated by face-to-face interviews including the semi-structured interview Schedules for Clinical Assessment in Neuropsychiatry (SCAN)(33) by trained medical doctors (8). Probands were asked to permit a contact to their adult children and siblings.

Participants

Participants were recruited as healthy first-degree relatives (adult children or siblings) of the probands described above. Individuals meeting the inclusion criteria and none of the exclusion criteria were enrolled in the trial, Table 1.

Assessments

The first part of the assessment was a telephone interview of the potential participants. The individuals eligible were scheduled to meet at the clinic on two different days both before and following four weeks of intervention. On the first day the participants gave written informed consent after details of the trial were explained. Diagnoses were ascertained by the SCAN interview and the structured Clinical Interview for DSM-IV Axis II Personality Disorders (4).

Further assessment included information on family history of psychiatric disorders, ratings of mood using the 17-item Hamilton Depression Rating Scale (HAM-D)(6) and 14-item Hamilton Anxiety Scale (6), various socio-demographics, height, weight, routine blood tests, and, a pregnancy test for women. The DEX-CRH test was performed at entry and following four weeks of intervention. Furthermore, following four weeks of intervention blood was drawn for measurements of plasma escitalopram, and the UKU Side Effect Rating Scale (22) was applied by the principal investigator.

Interventions

The participants were randomized to self-administer a single dose of either escitalopram 10 mg or placebo for four weeks. The manufacturer provided escitalopram and placebo tablets. The tablets were identical in appearance, color, smell, and solubility allowing for blinding of the assignment t intervention or placebo. An independent pharmacist packed the identically appearing blister packages containing escitalopram or placebo and then sealed, and numbered the packages according to a randomization list provided and concealed by the Copenhagen Trial Unit (CTU). O completion of the four weeks of intervention participants entered a five-day down-titration period to nil medication. Compliance to the protocol was sought by making weekly telephone calls to the enrolled participants. The participants were asked at the end of the trial, if they had missed taking any tablets.

Randomization

CTU performed the centralized computerized randomization 1:1 by telephone to secure adequate allocation sequence generation and allocation concealment. Randomization was stratified in block of 6, by age (18–31 and 32–60 years), and sex. Only the data manager knew the block size.

Blinding

All trial personnel and participants were blinded to the packaging of the trial drug, and blinding was maintained throughout monitoring, follow-up, assessment of outcomes, data management, data analyses, and conclusions drawn (12). At the assessment after four weeks intervention, each participant and the principal investigator (UK) made a guess as to which intervention the participant had received. The agreement between the actual intervention and the guesses was estimated to assess the degree to which blinding had been demasked, thus κ: < 0 no; 0.0-0.20=slight; 0.21-0.40=some; 0.41-0.60=moderate; 0.61-0.8 =substantial; 0.81-1.00=almost complete demasking.

Analysis of plasma escitalopram

The extraction and quantitation of escitalopram was carried out on an ASPECXL combined with a high-pressure liquid chromatography system, both from Gilson, Villiers le Bell, France. Lower and upper limits of quantitation were 10 nmol/l and 3,600 nmol/l. The interassay coefficients of variation ranged from 5.5% to 8.4% and trueness ranged from 93.2% to 103.0% within the measurement range.

Change in hormone responses to the DEX-CRH test

Cortisol and ACTH levels in response to the DEX-CRH test were measured before and after four weeks of intervention. The DEX-CRH test was performed according to international standards with a few minor technical modifications (25). A trained bio-technician and trained medical students conducted the tests under the supervision of the principal investigator.

Analyses of cortisol and ACTH

Hormones were analyzed at the Department of Clinical Biochemistry, Rigshospitalet. Plasma cortisol was measured using a competitive electro chemiluminescense immuno assay (ECLIA) (Roche Diagnostica Cortisol) and Modular analytics E170 (Roche). Lower and upper limits of quantitation were 1.0 and 17,500 nmol/l. The interassay coefficients of variation were 4.7% and 5.6% at 116 and 968 nmol/l, respectively. Plasma ACTH was measured using a sandwich chemiluminescense immunometric method (ACTH, Immulite Siemens DPC) and Siemens Immulite 2000. Lower and upper limits of quantitation were 1.0 and 556 pmol/l. The interassay coefficients of variation were 7.6% and 6.1% at 7 and 106 pmol/l, respectively.

In accordance with Modell *et al*, cortisol and ACTH responses were calculated according to the trapezoidal rule as the total area under the curve (AUC_{total}) from administration of CRH at 15:00 to the last measure at 18:00 (25). The plasma cortisol (COR) BASAL was estimated as the mean of the baseline measurements before the administration of CRH. CorPEAK was estimated as the highest plasma cortisol measurement following CRH administration. The primary outcome, the change in plasma cortisol response AUC_{total} (Δ $CorAUC_{total}$), was calculated by subtracting $CorAUC_{total}$ at four weeks from the $CorAUC_{total}$ immediately before the initiation of the intervention. Similarly, Δ was calculated for ACTH AUC_{total}, CorBASAL, and CorPEAK.

Statistical methods

The sample size estimation and the pre-established data analysis plan have previously been described (20). The power calculations were hypothetical since the effect of SSRI on the DEX-CRH test in healthy has not been investigated in prior trials (20). Thus, the power calculation was merely guided by a previous case control study in which the difference between healthy with and without a family history of MDD was regarded as a possible relevant difference (25), reflecting the hypothesis that the increased cortisol response to the DEX-CRH test in individuals with a family

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history of MDD would decrease, as a result of the SSRI intervention, to the level of the cortisol response measured in healthy without a family history of MDD.

Data from all randomized participants were analyzed, including those with missing data on the DEX-CRH test. The primary outcome measure was not normally distributed, and could not be transformed into a normal distribution. Thus, the outcome in the intervention and the placebo groups were compared by the Mann-Whitney test. Effect sizes were calculated unadjusted and adjusted for design variables, including stratification variables (1) age, sex, HAM-D total score at entry, body mass index at entry, number of daily cigarettes, and concentration of escitalopram in plasma, if the univariate analyses of these factors had a p-value < 0.1 (2). Initially, the drug level measured in each participant was not included in the models as to keep the analyzers blinded. Lastly, after every other analysis had been done and conclusions drawn, analyses for the effect of drug-level were performed. Analyses were performed both on complete datasets, as well as datasets on all participants completed by multiple imputation analysis of missing data from the DEX-CRH test (SAS version 9.1) (20).

Results

Participants and non-participants characteristics

The probands (n=466) gave us permission to contact 359 first-degree relatives, who were the potential participants in the trial. The participant flow is shown in Figure 1. A total of 80 participants were included and randomized. The clinical and demographic characteristics of the participants can be seen in Table 2. The mean age of the non-participants was 37 (SD 11) years and 58% were women. The reasons for their non-participation are presented in Figure 1.

The success of blinding

The agreement between the actual intervention group and the guess was 'some' demasking (κ = 0.23 (0.01-0.45)) for the participants and 'slight' demasking (κ = 0.18 (0.00-0.40)) for the principal investigator.

Adherence to the intervention and adverse events

Two participants randomized to escitalopram were excluded from the trial prior to intervention: one man withdrew informed consent, and one woman developed skin rash necessitating glucocorticosteroid treatment. No participants left the placebo group, and 33 in the escitalopram group and 32 in the placebo group stated full compliance with the protocol. Six participants in the escitalopram group and seven in the placebo group stated that they missed taking one or two tablets. No severe adverse reactions, or serious adverse events occurred. Following four weeks of intervention, 56% of the participants in the placebo group and 46% of the participants in the escitalopram group, reported no side effects. Adverse events are listed in Table 3.

Plasma escitalopram

Blood was drawn from all 78 participants at follow up, but one test from the escitalopram group failed. The mean concentration of escitalopram was 50 nmol/l, SD 29 nmol/l, median 48 nmol/l, range <10 to 138 nmol/l, (n= 38). Two participants from the escitalopram group had undetectable plasma escitalopram, thus <10 nmol/l, one of which had stated missing the last two tablets prior to blood sampling. Plasma escitalopram was undetectable in all participants of the placebo group.

Cortisol and ACTH response in the DEX-CRH test

The two datasets for the DEX-CRH test were complete for 73 participants. Thus, two participants had no tests. Further, one woman and one male missed the baseline test due to schedule problems.

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The test following the intervention was missed by two males due to schedule problems and one male due to technical reasons.

There was no statistically significant difference of the primary outcome $\Delta CorAUC_{total}$ comparing the intervention and the placebo groups, ($p = .47$), (Table 4).

In univariate analyses, no statistically significant correlations were found between $\Delta CorAUC_{total}$ and the variables: age, sex, HAM-D, body mass index, and number of daily cigarettes at randomization (results not presented). We found no significant differences between the results of the complete-case analysis and the analysis done after multiple imputations (results not presented).

The correlation between plasma escitalopram and $\Delta CorAUC_{total}$ were analyzed in the escitalopram group. Increasing plasma escitalopram was significantly correlated with decreasing $\Delta CorAUC_{total}$, (Friedmanns rho = - .41 ($R^2 = 0.046$), $p = 0.01$).

Post-hoc explorative analyses

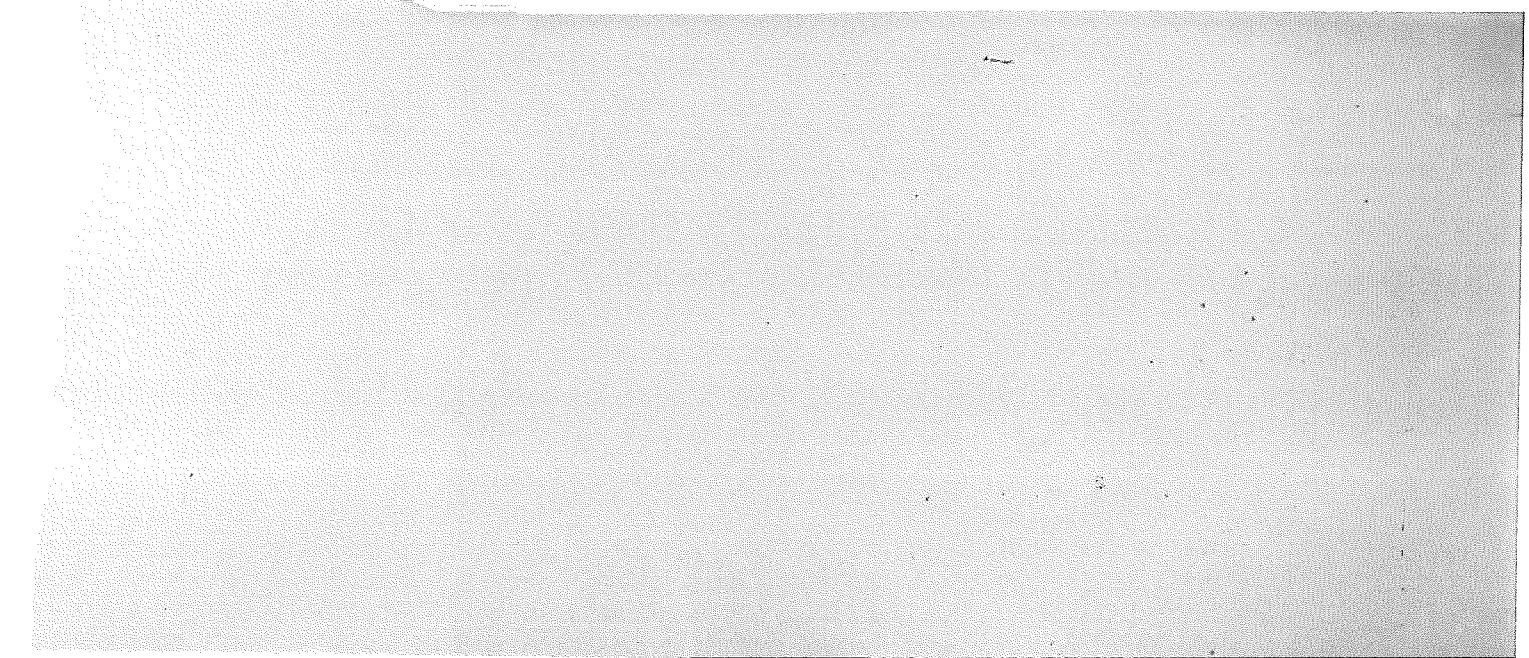
The escitalopram group and the placebo group did not separate significantly in analyses of Δplasma ACTH AUC_{total}, ΔCorBASAL, or ΔCorPEAK, Table 4. In additional analyses we found that the logarithm of AUC_{total} for plasma cortisol before and after the intervention followed a normal distribution with good approximation. Thus, the measure: ΔlogCorAUC = $\ln(CorAUC_{total.after}) - \ln(CorAUC_{total.before}) = \ln(CorAUC_{total.after} / CorAUC_{total.before}) = \ln(ratio)$, which has a normal distribution, was analyzed. The means of ΔlogCorAUC for escitalopram versus placebo did, however, not differ significantly, ($p = .49$).

There was a statistically significant interaction for ΔlogCorAUC between age and intervention group. Thus, the slope relating to age ΔlogCorAUC, (p = .024) differed significantly between the two intervention groups and the correlations between age and ΔlogCorAUC were R^2 = .07, (Pearson´s rho = - .27), for escitalopram and R^2 = .08, (Pearsons´s rho = .28) for placebo.

Data were moreover analyzed using mixed model effect analyses (results not presented) and no statistically significant difference between the intervention and the placebo group was found. In accordance with Modell *et al.* (25), a subgroup of 23 individuals with a PEAK cortisol concentration of 110 nmol/l or more in the DEX-CRH test at trial entry was analyzed. No statistically significant difference was shown on the $\Delta CorAUC_{total}$ for this subgroup, (p = .9). In addition, we analyzed the effect of escitalopram on $\Delta CorAUC_{total}$ for participants of the escitalopram group that had detectable escitalopram in plasma (n=36) versus placebo, but no statistically significant difference was found, (p = .69).

Discussion

The AGENDA trial is the first trial in which the effect of SSRI in healthy first-degree relatives of patients with depression has been investigated. Additionally, the AGENDA trial is the largest tria hitherto (n=80) in which the effect of SSRI is investigated in healthy individuals regardless of outcome (19). The main finding was that four weeks of intervention with escitalopram 10 mg/day compared with placebo had no statistically significant effect on neuroendocrine responses in the HPA-axis, as measured by $\Delta CorAUC_{total}$ in the DEX-CRH test, in healthy first-degree relatives o patients with MDD. Thus, our hypothesis that an intervention with escitalopram 10 mg would decrease the cortisol response in DEX-CRH test in healthy first-degree relatives of patients with MDD was not supported. Further, no statistically significant effect was found on any other



measure of the DEX-CRH test (Table 4). Post-hoc analyses showed that increasing levels of escitalopram tended to decrease the HPA-response in the DEX-CRH test and this effect increased with age.

Advantages of the trial

The AGENDA trial has several advantages. Firstly, the trial and the analyses were carried out as planned in advance and the completion and compliance in the trial was very high. Secondly, the registered diagnose of depression for the probands was verified by a face-to-face psychiatric research interview by trained medical doctors. The participants were assessed and diagnosed by validated and frequently used multi-dimensional methods. Thirdly, the participants were genetically homogeneous as all were ethnic Danes with European, mostly Danish, parents and grandparents. Fourthly, we used well established methods, e.g., the DEX-CRH test which is a sensitive, biological, objective test to detect increased HPA-function in humans (16;17). The response to the DEX-CRH test may be sensitive to age (16) and sex (21), and in our trial, stratification by these factors resulted in equal distributions in the two intervention groups. Fifthly, the participants were studied in a randomized clinical trial blinded in all phases including the statistical analyses and conclusion phase. The blinding was successful in relation to participants as well as researchers. Sixthly, the antidepressant effect of escitalopram is generally accepted (31). Further, the participants were subjected to four weeks of intervention thus including the interval in which clinical improvement has been reported in trials with patients with MDD (31).

Limitations

We have not compared healthy individuals with a family history of MDD to healthy individuals without a family history of MDD. However, the participants included in the present trial presented with values of $CorAUC_{total}$ in the initial DEX-CRH test before intervention, (Table 2 (12,005 ±

15,506 nmol/l x min/l)) that were higher than values found among healthy individuals without a family history of MDD in the study by Modell *et al.* (25) (7,773 ± 1,071 nmol/l x min/l) and approaching the values for healthy individuals with a family history of MDD in that study (15064 ± 3947 nmol/l x min/l), confirming that participants included in our trial were comparable to the participants with a family history of depression in the Modell study.

We cannot exclude that the dosage of 10 mg escitalopram was too low. However, this dosage has been suggested as the optimum dose for treatment of moderate depression (5) and it resulted in well-known adverse effects (Table 3).

Risk of errors

The risk of errors in trials falls in three major categories (10;11): 1) *Systematic error ('bias')*: We have minimized bias by using a randomized, age-and sex-stratified, comparison with blinding in all phases of the trial. 2) *Random error ('play of chance')*: We planned to include 80 participants due to resources, feasibility, and availability of the healthy first-degree relatives of patients with MDD studied in our group. Since no prior trials have investigated the effect of SSRI on healthy individuals, the power calculations were hypothetical and influenced by great uncertainty. Thus, we cannot exclude the possibility of overlooking a difference due to the play of chance. However, in the era of systematic reviews it has been questioned if the size of an individual trial still does matter. The results from any trial may contribute to the larger body of evidence despite arbitrary sample size calculations in the individual trial that may eventually prevent important trials from being conducted (13). 3) *Design errors*: These errors may include that some participants may not have reached sufficient levels of escitalopram in the blood in order to produce an effect on the HPA-axis. Our serum escitalopram concentrations were lower than in a study by Soegaard *et al.*

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(30), who found steady state plasma escitalopram concentrations of 63 ± 32 nmol/l for escitalopram 10 mg as compared to 50 ± 29 nmol/l in our trial. The low plasma levels in our trial may be a result of the fact that approximately 12 hours elapsed from taking the last tablet to blood sampling.

Generalizability, clinical implication and future studies

Our participants were healthy, ethnic Danes, with a parent or a sibling who was treated for depression in a hospital setting in Denmark. Our results may generalize to healthy Caucasians in general. To infer direct clinical implications from the results were not an aim of our trial, but effects by escitalopram 10 mg on the primary outcome for the HPA-axis function in healthy was not detected. Future studies may explore individuals in prodromal phases of depressive disorder or establish a run in period to optimize adherence to protocols. Further, the distinction between healthy participants with and without increased familial risk for MDD needs further exploration.

Interpretation

Considering advantages, disadvantages, risk of errors, and generalizability of the findings in this trial, it is likely that the results reflect reality. Thus, activation of the monoaminergic neurotransmitter systems by escitalopram does not seem to substantially affect the HPA-axis as measured by the DEX-CRH test in healthy individuals with a family history of depression. This finding seems to indicate that intervention with SSRI does not reduce the response to stress in first-degree relatives. Our finding is in accordance with recent data showing that restoration of HPA system dysfunction seems to be neither a necessary nor a sufficient determinant for an acute treatment response in depressed patients (28). Taken together these findings suggest that dysregulation of the HPA-axis does not play a *primary* role in the mechanisms of action of SSRIs.

The HPA dysregulation seen in depressed patients may rather represent the down stream effects of other, more primary abnormalities as suggested by Manji *et al.* (15).

Conclusions

The AGENDA trial is the first to investigate the effect of a long-term intervention with escitalopram on serotonin-mediated HPA-axis responses in healthy first-degree relatives of patients with MDD. The results did not show a statistically significant difference in $\Delta CorAUC_{total}$ in the DEX-CRH test between escitalopram 10 mg and placebo given for four weeks. Further, the results showed large intra- and inter-individual differences in the response to the DEX-CRH test. Increasing drug levels of escitalopram tended to decrease the HPA-response in the DEX-CRH test and this effect increased with age.

Acknowledgements

We thank probands and participants for their collaboration. Ulla Knorr was supported by a fellowship from the Center for Pharmacogenomics, University of Copenhagen. The Danish Research Council, University of Copenhagen and the Lundbeck Foundation supported the trial. H. Lundbeck A/S supplied the trial drug and placebo free of charge. The foundations of Eli Larsen, Jeppe Juhl, Geert Jørgensen, Ivan Nielsen, and Arvid Nilsson gave unrestricted economical support. Roche Diagnostics and Siemens Healthcare Diagnostics supplied the reagents for measurements of plasma cortisol and plasma ACTH, respectively. Maiken Madsen, Gunnhild Helmsdal, MD, and Lotte Lykkegaard, MD assisted in the conductance of the DEX-CRH tests. Lisbeth Andreassen provided technical assistance in measurement of plasma hormones. Helen Gerdrup Nielsen assisted in organizing the trial. Nader Salas, CTU, designed the randomization system and Kristian Juul is thanked for being the GCP-coordinator of the trial.

Table 1. Criteria for inclusion and exclusion in the AGENDA trial.

Inclusion criteria	**Exclusion criteria**
Healthy individual of both sexes*	Somatic illness or other handicap, which made participation in the trial impossible
Offspring or sibling of an ethnic Dane, with a history of psychiatric in- or outpatient care with a diagnosis of major depressive disorder and who later had the diagnosis verified in a SCAN interview at the Department of Psychiatry Rigshospitalet, Denmark 2004-2009	Daily intake of drugs interfering with corticosteroids or escitalopram, including birth control pills or any kind of corticosteroids
Aged 18 – 60 years. Women were preferably in lutheal phase menstrual cycle at the time of randomization	Hypersensitivity to escitalopram, dexamethasone, or human corticotropin-releasing hormone
Born in Denmark	Former medical or psychological treatment for diseases in the affective or schizophrenic spectrum
European parents and grandparents	Current abuse of alcohol or psychotropic medication
Able and willing to sign informed consent	Pregnancy or breastfeeding

* A total of 6 participants with stable treated medical conditions were included: hypertensio arterialis (3), pancreatitis antea (1), hypothyroidism (1) and acne vulgaris (1)

Table 2. Clinical and demographic characteristics of participants at entry

Characteristic	Escitalopram (N = 41)	Placebo (N = 39)	All Participants (N = 80)
Age – yr, mean ± SD	32 ± 11	31 ± 11	32 ± 10
Women – N (%)	15 (37)	14 (36)	29 (36)
Proband was / – N (%)			
sibling	18 (44)	15 (39)	33 (41)
parent	23 (56)	24 (62)	47 (59)
Caucasian – (%)	100	100	100
Education – mean ± SD			
Years of school	11 ± 1	11 ± 1	11 ± 1
Years of further education	3 ± 2	3 ± 2	3 ± 2
Employment status – N (%)			
Employed	30 (73)	26 (67)	56 (70)
Student	11 (27)	11 (28)	22 (28)
Unemployed	0 (0)	2 (5)	2 (3)
Marital status – N (%)			
Single	15 (37)	23 (59)	38 (48)
Married or cohabiting*	26 (63)	16 (41)	42 (52)
First degree relatives of patient with a history of major depressive disorder – median (quartiles) **	1 (1;2)	1 (1;2)	1 (1;2)
Second degree relatives with a history of major depressive disorder – median (quartiles)	0 (0;1)	0 (0;1)	0 (0;1)
17-item Hamilton Depression Scale Score, – median (quartiles) (range)	1 (0;3) (0-7)	1 (0;3) (0-7)	1 (0;3) (0-7)
14-item Hamilton Anxiety Scale Score, – median (quartiles) (range)	1 (0;2) (0-9)	1 (0;2) (0-6)	1 (0;2) (0-9)
Beck Depression Inventory, 21-item, depression – median (quartiles)	2 (0;4)	2 (0;3)	2 (0;5)
Beck Depression Inventory, 14-item, anxiety – median (quartiles)	1 (0;4)	2 (0;3)	1 (0;3)
Body Mass Index – kg/m^2, mean ± SD	25 ± 4	26 ± 5	26 ± 4
Numbers of daily cigarettes - median (quartiles)	0 (0;11)	0 (0;10)	0 (0;10)
Package years – median (quartiles)	1 (0;10)	2 (0;7)	1.75 (0;8)
Daily medicine – N (%)	2 (5)	4 (10)	6 (8)
Plasma cortisol AUC_{total} - nmol/l x min/l, mean ± SD, median (quartiles)	9045 ± 12829 4691 (2864;8277)	15126 ± 17542 9974 (2549;18336)	12005 ± 15506 5095 (2669;13833)
Plasma ACTH AUC_{total} - pmol/l x min/l, mean ± SD, median (quartiles)	324 ± 272 255 (209;304)	365 ± 197 306 (233;426)	343 ± 239 263 (215;263)
Plasma cortisol BASAL - nmol/l, mean ± SD, median (quartiles)	15 ± 15 13 (8;17)	24 ± 37 15 (10;20)	19 ± 28 14 (9;18)
Plasma cortisol PEAK - nmol/l, mean ± SD, median (quartiles)	90 ± 124 41 (22;82)	137 ± 153 86 (19;191)	112 ± 140 52 (20;136)

Notes: Two smoked cannabis more than two months prior to the investigation. Three were previously abusing alcohol. One participant had generalized anxiety.
* Eight were living with their parents.
** quartiles reported, are the 25 and 75 quartiles
*** There was no statistically significant difference between the escitalopram and the placebo group for any of the hormone measures. AUC_{total} = Area under the curve after administration of CRH corrected for baseline equivalent, BASAL = mean of five measurements at the baseline after pre-treatment with dexamethasone 1.5 mg and before the administration of CRH, PEAK = the highest measurement following CRH administration.

Flowchart for the AGENDA trial

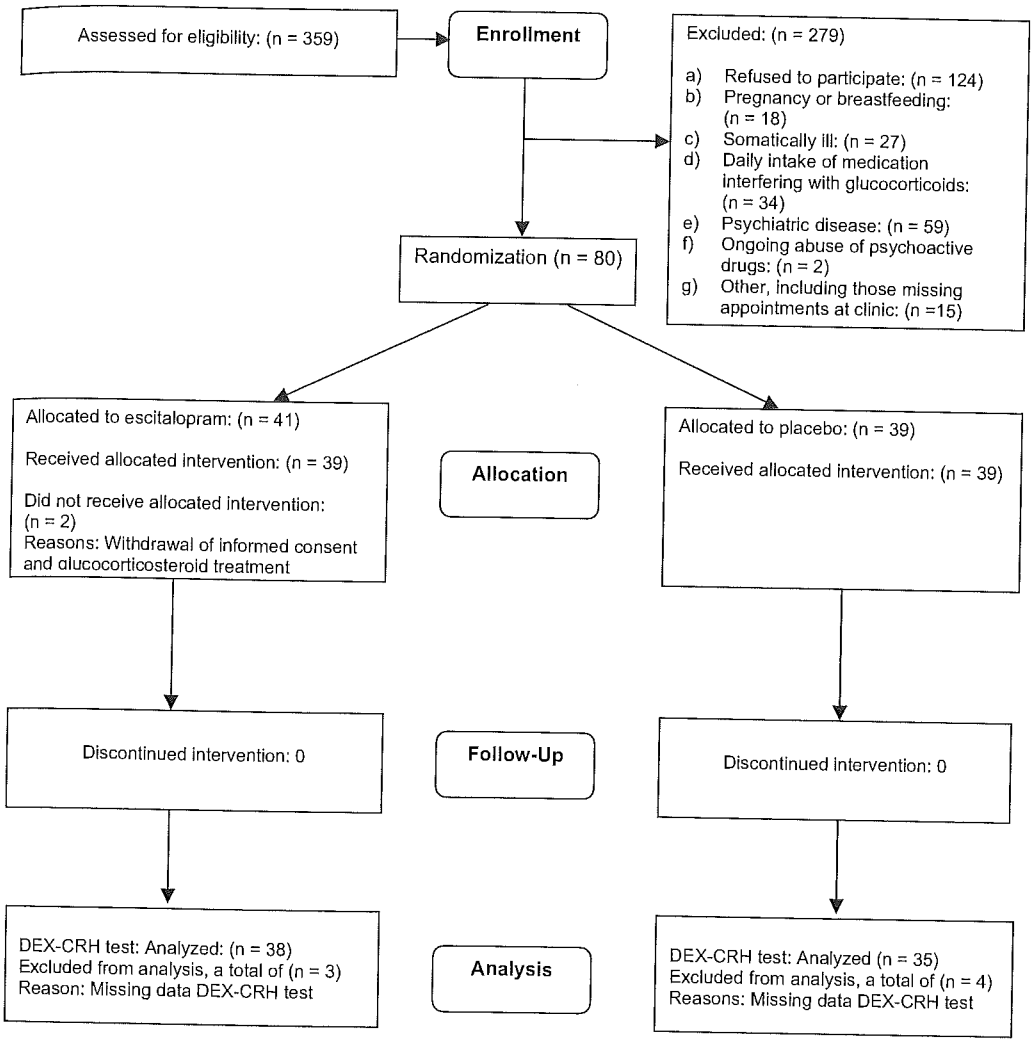

Table 3. Assessed adverse events by the UKU Side Effect Rating Scale for 78 healthy first degree relatives of patients with a history of major depressive disorder following four weeks of intervention by escitalopram 10 mg (n = 39) or placebo (n = 39) in the AGENDA trial

Adverse events	Escitalopram %	Placebo %	p (χ^2)
Restlessness	15	23	.39
Insomnia	5	23	.02
Tremor	3	3	1.00
Nausea	10	10	1.00
Diarrhoea	10	3	.17
Sweating	15	10	.50
Less libido	18	5	.08
Erective dysfunction (men)	13	3	.09
Ejaculating problems (men)	28	3	.002
Orgasmic dysfunction	28	0	.000
Headache	3	3	1.00

Table 4. The distributions of the primary outcome measure and other characteristics of plasma cortisol and plasma ACTH in the combined DEX-CRH test in 73 healthy first degree relative of patients with a history of major depressive disorder, in the escitalopram 10 mg group (n = 38) and the placebo group (n = 35).

Quantity	Group (N)	Mean (SD)	Median	Minimum value	Maximum value	Interquartile range	p [b)]
Δ plasma cortisol AUC_{total} [a)]	Escitalopram	1675.1 (13001)	606.6	-40895.6	47913.8	8782.6	.47
	Placebo	1170.5 (17910)	-200.0	-44680.2	56859.7	7064.2	
Δ plasma ACTH AUC_{total}	Escitalopram	25.1 (158)	-0.08	-392.0	653.0	67.1	.23
	Placebo	-6.48 (255)	-10.7	-750.0	743.0	108.0	
Δ plasma cortisol BASAL	Escitalopram	0.461 (13.5)	-0.345	-25.4	72.9	4.60	.57
	Placebo	5.17 (48.4)	0.340	-363	84.1	5.49	
Δ plasma cortisol PEAK	Escitalopram	3.96 (124)	-3.92	-348	356	80.0	.61
	Placebo	1.76 (131)	1.23	-348	422	69.7	

Δ was the difference between the measurement of plasma cortisol and ACTH after and before four weeks of intervention with escitalopram 10 mg or placebo for:
AUC_{total} = Area under the curve after administration of CRH corrected for baseline equivalent,
BASAL = mean of five measurements at the baseline after pre-treatment with dexametasone 1.5 mg and before the administration of CRH,
PEAK = the highest measurement following CRH administration,
[a)] Δ plasma cortisol AUC_{total} was the primary outcome measure
[b)] p of Mann-Whitney test comparing the two distributions which did not follow normal distributions (Shapiro Wilkes test).

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Escitalopram versus placebo on personality in healthy first-degree relatives of patients with depression: the randomized, blinded AGENDA trial

ORIGINAL ARTICLE

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Neuroticism seems to reflect an enduring vulnerability to major depressive disorder (MDD) (Kendler et al., 2006). This may partly reflect shared genetic risk factors and most of the genetic risk for MDD expressed via personality is captured by neuroticism, with a modest amount by conscientiousness, and small amounts by openness, extroversion, and agreeableness (Huezo-Diaz et al., 2005;Kendler et al., 2009). When neuroticism decreases in patients with depression who are treated with antidepressants, it has been difficult to clearly distinguish the treatment effect on neuroticism from the treatment effect on the depressive disorder, as remission of depressive symptoms is associated with partial normalization of neuroticism (Tang et al., 2009). Decrease in neuroticism scores during paroxetine treatment of patients with MDD, even after controlling for depression improvement, has been observed in a large group of depressed patients (Tang, DeRubeis, Hollon, Amsterdam, Shelton, and Schalet, 2009). Thus, it is possible that response to selective serotonin re-uptake inhibitors (SSRIs) may be mediated at least partly via a decrease in neuroticism (Quitkin, 1999;Tang, DeRubeis, Hollon, Amsterdam, Shelton, and Schalet, 2009). Higher neuroticism is associated with higher thalamic serotonin binding (Takano et al., 2007). Furthermore, a recent study from our group has suggested that familial risk of depression and neuroticism interact in their relation to the degree of specific serotonin transporter binding (Frokjaer et al., 2009).

Two randomized trials have investigated the effect of SSRI on behavior and aspects of personality with some relation to neuroticism in healthy participants without a family history of MDD. Knutson et al found that four weeks intervention with paroxetine 20 mg/day (N = 26) versus placebo (N = 25) significantly increased social affiliation and decreased negative affect (Knutson et al., 1998). Tse et al found that two weeks intervention with citalopram 20mg/day (N = 11) compared with placebo (N = 9) induced a statistically significant increase in self-directedness (Tse et al., 2001). Results from these trials suggest that SSRI administration may

affect personality even in the absence of clinical depression. Furthermore, no effect of SSRI on mood in healthy individuals have been shown (Arce et al., 2007;Harmer et al., 2006;Nemoto et al., 2003;Tse et al., 2002). Results from a number of studies, although not all (Rothen et al., 2009) have suggested increased levels of neuroticism in healthy first-degree relatives of patients with MDD compared to healthy individuals without a family history of MDD (Christensen et al., 2006). However, no trial has investigated the effect of SSRIs on neuroticism and other personality dimensions in healthy individuals with a family history of MDD (Knorr et al., 2010a).

Thus, to examine the effect of a SSRI on neuroticism excluding an effect on depression, we recruited healthy first-degree relatives of patients with MDD for the AGENDA (**A**ssociations between **G**ene-polymorphisms, **E**ndophenotypes for **D**epression and **A**ntidepressive Intervention) trial. The trial is the first to investigate the effect of a four week self-administered daily SSRI versus placebo on personality traits, as measured with the NEO-PI-R and EPQ, in healthy individuals (Knorr et al., 2009). We tested the hypothesis that intervention with a SSRI for a month compared with placebo decreases neuroticism scores in healthy first-degree relatives of patients with MDD.

1. Methods

1.1. Study design

The AGENDA trial was investigator initiated and designed. It was conducted as a participant-, investigator-, observer-, and data-analyst-blinded trial. During the trial the participants received either escitalopram 10 mg/day or placebo for a period of four weeks. The trial was conducted from July 2007 until July 2009 at the Department of Psychiatry, Rigshospitalet, Copenhagen University Hospital, Denmark and Ulla Knorr was supported by a fellowship from the Centre

for Pharmacogenomics, University of Copenhagen, Denmark. The regional ethics committee for the greater Copenhagen area (H-KF-307413), the Danish Data Protection Agency (2006-41-6737), and the Danish Medicines Agency (2612-3162) approved the protocol. The trial has the EudraCT number 2006-001750-28 and was registered at www.clinicalrials.gov as NCT 00386841 (AGENDA). The trial was conducted and monitored in accordance with the International Conference on Harmonization for Good Clinical Practice guidelines(2010) and the Declaration of Helsinki 2002, www.wma.net/e/policy/b3.htm. An independent data monitoring and safety committee was established to further ensure the safety of the participants, should the need have occurred for early stopping. All participants gave written informed consent. The detailed study protocol of the AGENDA trial was published ahead of study completion and the changes in neuroticism scale scores on the NEO-PI-R and the EPQ were pre-defined as secondary outcomes (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009). Results on the primary outcome change in the area under the curve (AUC) for cortisol measurements during the combined dexamethasone-corticotropin releasing hormone test have been submitted elsewhere (Knorr et al., 2010b)

1.2. Probands and participants

The selection of probands and participants have previously been described in details (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009). Probands were patients with a diagnosis of MDD from psychiatric hospital in- or out-patient contact in Denmark who participated in ongoing studies at the Department of Psychiatry, Rigshospitalet, Denmark. Their diagnoses were validated by face-to-face interviews including the semi-structured interview Schedules for Clinical Assessment in Neuropsychiatry

for

(SCAN) by trained clinicians (Wing et al., 1990). Probands were asked to permit a contact to their adult children and/or siblings who were the eligible participants for the AGENDA trial. The probands (n = 466) gave written permission to contact 359 first-degree relatives, who were the potential participants in the trial. The participant flow is shown in Figure 1.

Individuals of either sex, aged 18 – 60 with Danish ethnicity (i.e., born in Denmark, with Danish parents and European grandparents) were eligible for the trial. Ethnicity was used to get a genetically homogeneous sample. We excluded individuals with somatic illnesses or a handicap that made participation in the trial impossible while six individuals with stable, treated milder medical conditions were included: hypertensio arterialis (three), pancreatitis antea (one), hypothyroidism (one), and acne vulgaris (one). Furthermore, we excluded individuals with a daily intake of drugs interfering with corticosteroids or escitalopram (cipralex), including birth control pills or any kind of corticosteroids, and individuals who were allergic to the study drug or placebo. Additionally, former medical or psychological treatment for diseases in the affective or schizophrenic spectrum and current abuse of alcohol or psychotropic medication led to exclusion. Women who were trying to conceive, or who were pregnant or breastfeeding were excluded. Women were preferably in the luteal phase of the menstrual cycle at the time of randomization. Women taking birth control pills were instructed to discontinue these six weeks prior to entering the trial. Furthermore, all women were carefully instructed to use double barrier birth control methods and pregnancy tests were performed both before and after the intervention (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009).

1.3. Trial procedures

Escitalopram and placebo tablets were identical in appearance, color, smell, taste and solubility allowing for blinding of the assignment to intervention or placebo. H. Lundbeck A/S provided

identically appearing blister packages containing escitalopram or placebo. An independent pharmacist then packed, sealed, and numbered the drug packages according to a randomization list provided and concealed by the Copenhagen Trial Unit (CTU). The participants were randomized to self-administer a single dose of either escitalopram 10 mg or placebo each evening for four weeks. On completion of four weeks of double-blind intervention participants entered a five-day blinded down-titration period to nil medication. Adherence to the protocol was sought by making weekly telephone calls to the enrolled participants. Following four weeks of intervention the participants were asked, how adherent they had been to the protocol, and if they had missed taking any tablets. The sample size was estimated according to the primary outcome as previously described (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009). CTU performed the centralized computerized randomization by telephone to secure adequate allocation sequence generation and allocation concealment.(Wood et al., 2008) Randomization was stratified in blocks by age (18-31 years and 32-60 years) and sex. Only the data manager knew the block size, which was six. Participants were randomized 1:1 to receive either escitalopram 10 mg or placebo. Randomization was done immediately after a face-to-face interview including the SCAN-interview at the first scheduled appointment at the clinic establishing that a participant fulfilled all the inclusion criteria and none of the exclusion criteria. All trial personnel and participants were blinded to the packaging of the study drug, and blinding was maintained throughout monitoring, follow-up, data management, assessment of outcomes and data analyses. The randomization code was not broken until all data had been analysed and conclusions drawn. The blinding was successful and has previously been described (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009).

1.4. Assessments

The first part of the assessment was a telephone interview, and eligible individuals were scheduled to meet at the clinic on two different days both before and following four weeks of intervention. On the first day of examination the participants gave written informed consent after details of the trial were explained. Diagnoses were ascertained by the SCAN interview and the structured Clinical Interview for DSM-IV Axis II Personality Disorders.(1997). Further assessment included information on family history of psychiatric disorders, ratings of mood using the 17-item Hamilton Depression Rating Scale (HAM-D) (Bech et al., 1986), and the 14-item Hamilton Anxiety Scale (Bech, Kastrup, and Rafaelsen, 1986), self rated depressive symptoms by Beck Depression Inventory, 42-items,(BECK et al., 1961), various socio-demographics, height, weight, routine blood tests, and, a pregnancy test for women. Furthermore, blood was drawn for measurements of plasma escitalopram, and the UKU Side Effect Rating Scale(Lindstrom et al., 2001), was applied by the principal investigator after four weeks of intervention.

1.5. Assessment of neuroticism

The personality dimension neuroticism was assessed by the Danish version of Eysenck Personality Questionnaire (EPQ) (Eysenck HJ et al., 1975;Skovdahl-Hansen H, 2004), and the Revised Neuroticism-Extroversion-Openness-Personality Inventory (NEO-PI-R) (Costa, Jr. et al., 1997). The EPQ comprises 101 yes-no items that measure the broad dimensions of neuroticism, extroversion, and psychoticism. NEO-PI-R is a 240-items inventory that evaluates the broad personality dimension of neuroticism, extraversion, openness, agreeableness, and conscientiousness. The score on each of the five broad dimensions is derived by adding the scores

from the assessments of six constituent personality traits (facets). The respondent answers the statements on a 5-point Likert scale from 'disagree very much' to 'agree very much'. Both EPQ and NEO-PI-R were applied before (T0) and following four weeks of intervention (T4).

1.6. Measure of plasma-escitalopram

Escitalopram was extracted and quantitated was carried out on an ASPEC XL combined with a HPLC system, both from Gilson, Villiers le Bell, France. Lower and upper limits of quantitation were 10 and 3600 nmol/l, respectively. Imprecision ranged from 5.5% to 8.4% and trueness ranged from 93.2% to 103.0% within the measurement range. Extraction recovery was 38% and carry-over was less than 1%.

1.7. Statistical methods

The data analyses planned for the secondary outcomes were described in a pre-established statistical analysis plan (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009). The null-hypotheses to be tested were that there would be no difference between the two intervention arms with regard to changes in neuroticism assessed by the EPQ and NEO-PI-R. The outcomes were changes in personality scores calculated as the score at T4 minus the score at T0 for the individual participants. Firstly, independent samples t-tests were used to compare change scores in the escitalopram and placebo groups for NEO-PI-R neuroticism and EPQ neuroticism. Secondly, adjustments were planned to be conducted for age, sex, years of education, and concentration of escitalopram in plasma in a general linear model if these variables were associated with change in neuroticism at the 0.1 level of significance (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009).

2. Results

A total of 80 participants were randomized.The clinical and demographic characteristics of the participants can be seen in Table 1. The mean age of non-participants was 37 (SD 11) years and 58% were women. The reasons for non-participation are presented in Figure 1. A statistically significant correlation was found between EPQ neuroticism and NEO-PI-R neuroticism reported at entry (Pearsson = .80; $P < .0005$). No severe adverse reactions or serious adverse events were obseved. The side effect measure UKU total for participants of the escitalopram and placebo groups did not differ significantly at four weeks (Table 1). However, sexual adverse

effects showed a statistically significant increase and insomnia showed a statistically significant decrease in the escitalopram group compared with the placebo group(Knorr, Vinberg, Hansen, Klose, Feldt-Rasmussen, Hasselstrom, Hilsted, Winkel.P., Wetterslev, Gluud, Gether, and Kessing, 2010b).

2.1. Effects on neuroticism

The dataset was complete with the exception of one man and one woman in the escitalopram group, who left the trial prior to the intervention, and two men in the placebo group in whom data collection failed for both EPQ and NEO-PI-R in one and for EPQ in another. The change (T4-T0) in reported neuroticism scores for participants who took escitalopram compared with placebo participants showed no statistically significant difference, NEO-PI-R ($P = .09$) and EPQ ($P = .73$) (Table 2). No stastistically significant correlations were found between change in neuroticism measured using EPQ or NEO-PI-R, and age, sex, years of education, or plasma escitalopram. Post hoc analyses showed no statistically significant correlations between: change in EPQ neuroticism and BDI-21 at entry (rho = - .26; $P = .06$), change in EPQ neuroticism and HAM-D at entry (rho = .12; $P = .32$), change in NEO-PI-R neuroticism and BDI-21 at entry (rho = - .10; $P = .38$), and change in NEO-PI-R neuroticism and HAM-D at entry (rho = - .05; $P = .69$). Furthermore, no statistically significant differences were shown in changes in EPQ extraversion ($P = .24$), EPQ psychoticism ($P = .96$), NEO-PI-R extraversion ($P = .90$), NEO-PI-R openness ($P = .33$), and NEO-PI-R conscientiousness ($P = .07$) between escitalopram and placebo participants. However, a statistically significant difference was found in the change in NEO-PI-R agreeableness between escitalopram (mean; SD) 2.38; 8.09 and placebo -1.32; 7.94 ($P = .046$) (Table 2).

3. Discussion

The results of the AGENDA trial did not support our hypothesis that a four-week long intervention with escitalopram as compared with placebo would decrease EPQ or NEO-PI-R neuroticism scores in healthy first-degree relatives of patients with MDD. Furthermore, none of the other dimensions of personality measured by EPQ or NEO-PI-R were significantly affected by the intervention, except for changes on the agreeableness dimension of the NEO-PI-R. We found no significant correlation between change in neuroticism during intervention, and age, sex, education or plasma escitalopram concentration, respectively.

Results from a recent placebo-controlled trial in patients with major depression suggest that the SSRI paroxetine has a specific effect on personality traits of neuroticism and extraversion that is distinct from its effect on depression (Tang, DeRubeis, Hollon, Amsterdam, Shelton, and Schalet, 2009). On the other hand, another study found that reductions in neuroticism correlated with improvement in depression in response to treatment with a SSRI (Quilty et al., 2008). Our results show that escitalopram has no major direct effect on (mean; SD) neuroticism. Regarding our finding of a significant effect of SSRI on agreeableness, the result ($P < .046$) was not significant when considering the multiple significance testing of the many outcomes of the trial. Furthermore, agreeableness has not been shown to be significantly affected by SSRI treatment (flouoxetine) in a study of depressed patients (N = 53) (Du et al., 2002).

3.1. Limitations

It is a limitation that we have not compared healthy individuals with a family history of MDD to healthy individuals without a family history of MDD. Further, a large number of women were excluded from our trial due to oral contraceptives and pregnancy, thus the trial population is characterized by an overrepresentation of men. We cannot exclude that the dosage of 10 mg

escitalopram was too low although this has been suggested as the optimum dose for treatment of moderate depression (Bech et al., 2006). Even though the participants received weekly phone calls to optimise adherence, several of the participants in the escitalopram group were found to have low plasma escitalopram concentrations. We considered using a higher dosage, but escitalopram 20 mg daily might have given more adverse effects, eventually jeopardizing blinding and adherence, thus we decided to use 10 mg daily. This dose of escitalopram resulted in well-known adverse effects, such as sexual adverse effects, as described in a prior paper from the study (Knorr, Vinberg, Hansen, Klose, Feldt-Rasmussen, Hasselstrom, Hilsted, Winkel.P., Wetterslev, Gluud, Gether, and Kessing, 2010b).

We planned to include 80 participants due to resources and availability of the healthy first-degree relatives of patients with MDD, as previously described (Knorr, Vinberg, Klose, Feldt-Rasmussen, Hilsted, Gade, Haastrup, Paulson, Wetterslev, Gluud, Gether, and Kessing, 2009), The power calculations were very hypothetical since the Agenda trial is the first trial to investigate the effect of SSRI in healthy with a family history of MDD. However the AGENDA trial is the largest trial (N=80) on healthy regarding any outcome, as shown in a recent review from our group (Knorr and Kessing, 2010a). We found a tendency for escitalopram to reduce neuroticism when measured by the NEO-PI-R, but the opposite tendency when neuroticism was measured by the EPQ. Thus, it may not seem likely that our results are due to type II errors and that a larger sample would have changed the results. Furthermore, neuroticism reported by EPQ and NEO-PI-R was closely correlated.

3.2. Generalizability

To increase the chances of detecting an effect of escitalopram versus placebo we chose to include healthy individuals at increased risk of developing depression (i.e., with a first-generation family

history of depression) since such individuals are more likely to present with neuroticism as previously shown in a study from our group (Vinberg et al., 2007) and in other studies (Lauer et al., 1998). Since we found no effect of escitalopram in this high-risk group, we believe that the finding can be generalized also to healthy Caucasians without a family history of depression.

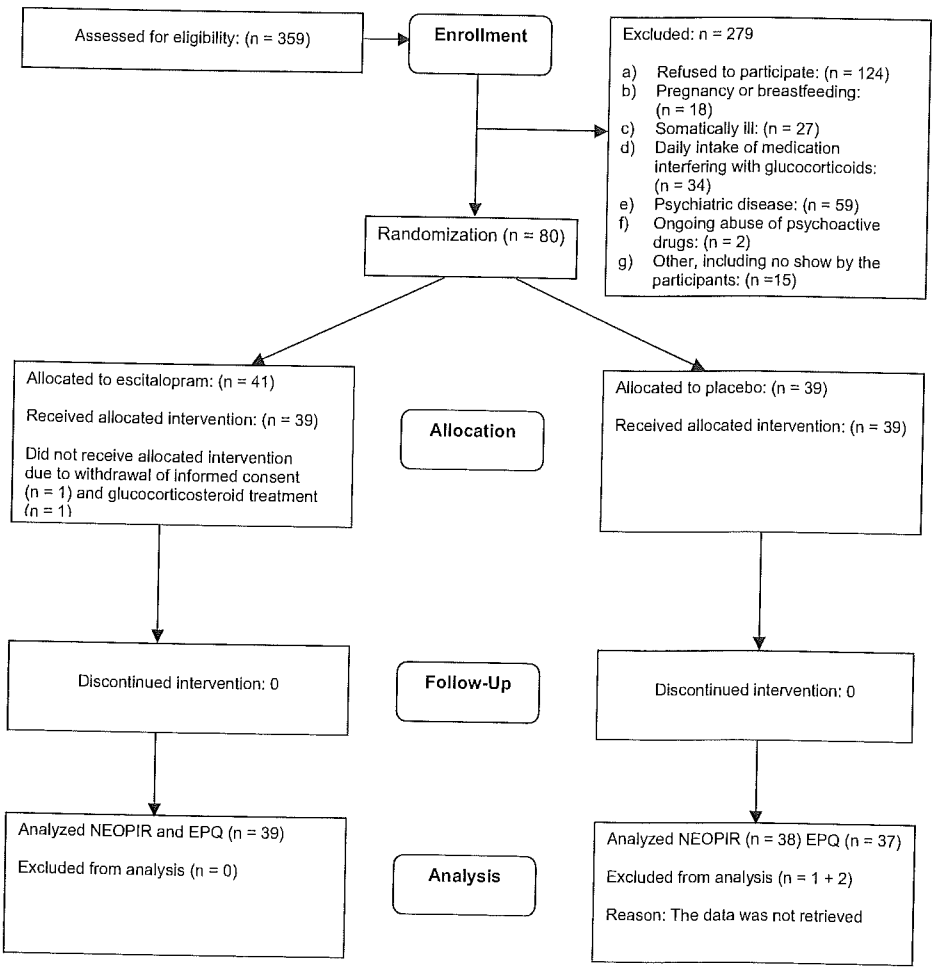
3.3. Perspectives

Studies of healthy first-degree relatives offer an excellent opportunity to determine whether personality traits represent a premorbid risk factor for subsequent onset of mood disorders or whether personality deviances are a consequence of mood episodes (Christensen and Kessing, 2006). Future studies may explore the suggested serotonergic link between the personality dimension agreeableness. If the finding of changes in agreeableness is replicated, it may lead to the hypothesis that SSRI do not directly modulate mood but rather mediate a different self-perception captured by changes in the scores of the facets of the personality dimension of agreeableness, which are trust, straightforwardness, altruism, compliance, modesty and tender mindedness.

4. Conclusions

The AGENDA trial is the first to investigate if an antidepressant intervention has an effect on neuroticism in healthy participants. Treatment with escitalopram during four weeks did not affect neuroticism scores in a relatively large group of healthy individuals with a first-degree relative with MDD. Post-hoc analyses, nevertheless, revealed that treatment with escitalopram compared with placebo might increase the NEO-PI-R personality trait agreeableness. The latter finding should be explored in future studies.

Figure 1. Flowchart for The AGENDA Trial



A randomised trial of the effect of escitalopram versus placebo on cognitive function in healthy first-degree relatives of patients with depression

FAST TRACK

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ABSTRACT

Background Selective serotonin receptor inhibitors (SSRI) may affect cognition in depressed patients, but it is unclear if the effect is directly on cognitive function or is secondary to the effect of SSRIs on depressive symptoms. It has not previously been tested whether treatment with a SSRI has a beneficial or detrimental effect on cognition in healthy individuals with a genetic liability for depression.

Aims To evaluate the effect of the SSRI escitalopram on cognitive function in healthy first-degree relatives of patients with major depressive disorder.

Method A total of 80 participants were randomised to escitalopram (10 mg/day) ($n = 41$) versus placebo ($n = 39$) for 4 weeks (trial registration NCT 00386841). Neuropsychological tests were applied at entry (T0) and at four weeks (T4). The main outcome measure was calculated as the change (T4-T0) in the general cognition score, which was the standardised mean of 13 test measures. Finally changes in four cognitive domain factors were calculated and compared.

Results Mean change in the general cognition score was not significantly increased with escitalopram compared with placebo, ($P = 0.37$), neither was the mean change of the scores for the four cognitive domain factors nor any of the specific tests. In univariate analyses no statistically significant correlations were found between change in the general cognitive score and the variables age, sex, Hamilton depression score 17 items, Danish Adult Reading Test-45, and plasma escitalopram levels, respectively.

Conclusions Treatment with escitalopram does not seem to either improve or impair cognitive function in healthy first-degree relatives of patients with depression. Improvement in cognitive function after treatment with SSRIs may be related to the effects on depression symptoms

Knorr U

Declaration of interest UK, AG, PW, JW, CG, and UG none. MV and LVK have been consultants for Bristol-Myers Squibb, Eli Lilly, Lundbeck, AstraZeneca, Servier and Jannsen-Cilag.

Trial registration

Local Ethics Committee: H-KF 307413.

Danish Medicines Agency: 2612-3162.

EudraCT: 2006-001750-28.

Danish Data Agency: 2006-41-6737.

www.clinicaltrials.gov: NCT 00386841 (AGENDA)

Key words

Cognitive function, healthy, trial, placebo-controlled, escitalopram, high risk, major depressive disorder

Introduction:

In depression a wide range of cognitive deficits is a consistent finding.[1] Cognitive function is a predictor of the functional and psychosocial burden of illness in major depressive disorder (MDD) and consequently a pertinent candidate predictor of treatment response.[2] With recovery from MDD, abnormalities in cognitive function tend to normalise but cognitive impairment is seen both in recovered patients and in healthy first-degree relatives of patients with MDD.[3-5] The diversity of symptoms in MDD suggests that many areas of the brain are involved in the pathophysiology of the disorder. The serotonin transporter is expressed abundantly in the raphe nucleus and in the limbic system which may be the main site of action for selective serotonin reuptake inhibitors (SSRI).[6] It is, however, not clear whether treatment with SSRIs result in a direct improvement of cognition or whether the effect of SSRIs on cognitive function is secondary to the effect of SSRIs on depressive symptoms. A neuropsychological hypothesis of antidepressant drug action suggests that, at the neuropsychological level, antidepressants work by re-mediating negative affective biases in depression and anxiety and that these actions occur relatively quickly following drug administration.[7-9] To disentangle the effect of antidepressant treatment from the effect of recovery from the depressive disorder per se, we investigated the effect of an SSRI on cognitive function in healthy first-degree relatives of patients with MDD. We hypothesised that four weeks of treatment with escitalopram would improve cognitive function compared with placebo.

Methods and Materials

The AGENDA (**A**ssociations between **G**ene-polymorphisms, **E**ndophenotypes for **D**epression and **A**ntidepressive Intervention) trial was designed as a participant, investigator, observer, and data-analyst-blinded randomised trial in which participants received either escitalopram 10 mg/day or matching placebo for a period of four weeks. The trial was conducted from July 2007 until July

2009 at the Department of Psychiatry, Rigshospitalet, University Hospital of Copenhagen, Denmark, as part of the Center for Pharmacogenomics, University of Copenhagen. The trial was conducted and monitored in accordance with the International Conference on Harmonisation for Good Clinical Practice guidelines[10] and the Declaration of Helsinki 2002 (www.wma.net/e/policy/b3.htm). The trial protocol including sample size estimation was published before completion of the trial.[11] Cognitive function was pre-defined as a secondary outcome measure of the AGENDA trial.

Assessments

The first part of the assessment was a telephone interview of the potential participants. The individuals eligible were scheduled to meet at the clinic both before and following four weeks of intervention. On the first day of examination the participants gave written informed consent after details of the trial were explained. Diagnoses were ascertained by the Schedules for Clinical Assessment in Neuropsychiatry (SCAN)[12] and the structured Clinical Interview for DSM-IV Axis II Personality Disorders.[13] Further assessment included information on family history of psychiatric disorders, ratings of mood using the 17-item Hamilton Depression Rating Scale (HAM-D),[14] and 14-item Hamilton Anxiety Scale,[14] various socio-demographics, height, weight, routine blood tests, and a pregnancy test for women. The neuropsychological test battery was applied on the same day as the interview and repeated following four weeks. The Side Effect Rating Scale by UKU-SERS-Pat[15] was applied by the principal investigator (UK) to assess side effects following four weeks of intervention.

Randomisation

Randomisation to one of the two intervention groups was done on the first day of examination immediately after it had been established that a participant fulfilled all the inclusion criteria and

none of the exclusion criteria. The Copenhagen Trial Unit (CTU) performed the centralised computerised randomisation by telephone to secure adequate allocation sequence generation and allocation concealment. Randomisation was stratified in blocks of six by age (18–31 years and 32–60 years) and sex. Only the data manager knew the block size. Participants were randomised 1:1 to receive either escitalopram 10 mg/day or placebo.

Blinding

All trial personnel and participants were blinded to the packaging of the trial drug, and blinding was maintained throughout monitoring, follow-up, data management, assessment of outcomes, and data analyses. The randomisation code was not broken until all data had been analysed and conclusions drawn, as previously suggessted.[16] At the assessment after four weeks intervention, every participant and the principal investigator (UK) made a guess as to which intervention the participant had received. A large proportion of the participants said, "I do not know" but were asked to give their best guess. The agreement between the actual intervention and the guesses was estimated to assess the degree to which blinding had been demasked, thus κ: < 0 no; 0.0-0.20 = slight; 0.21-0.40 = some; 0.41-0.60 = moderate; 0.61-0.80 = substantial; 0.81-1.00 = almost complete demasking.

Interventions

The participants were randomised to self-administer a single dose of either escitalopram 10 mg or matching placebo each evening for four weeks. Escitalopram and placebo tablets were identical in appearance, colour, smell, and solubility allowing for blinding of the assignment to intervention or placebo. H. Lundbeck A/S provided identically appearing blister packages containing escitalopram or placebo. An independent pharmacist then packed, sealed, and numbered the drug packages according to a randomisation list provided and concealed by the CTU. Adherence to the protocol

was sought by making weekly telephone calls to the enrolled participants. The participants were asked at the end of the trial how adherent they had been to the protocol, and if they had missed taking any tablets. On completion of four weeks of intervention participants entered a five-day blinded down-titration period to nil medication.

Neuropsychological tests

Cognitive functions were measured with neuropsychological tests at baseline and following four weeks of intervention. Descriptions of most of these tests may be found in "A compendium of neuropsychological tests" (Strauss et al., 2006),[17] and modifications are noted below. The 45-word Danish version of *National Adult Reading Test* (DART-45)[18] was used as a measure of intelligence. 13 measures from the other tests were subjected to factor analysis, yielding the following four factors:

Factor 1. Visuo-Motor/Visuo-Spatial function. This factor included five measures: *Trail Making A & B,* connecting numbers (A) and alternating numbers and letters (B); *Symbol Digit Modalities Test (SDMT),* a sensitive test requiring the subject to write numbers corresponding to each of nine symbols indicated in a coding key, in 90 seconds; *Block Design*,[19] a variant of the WAIS subtest with a score made up of the mean time in seconds to complete each of 12 designs with four blocks with red, white, and half red/white sides. *Rey-Osterrieth Complex Figure*, 3 minute free recall (copy score not included).

Factor 2. Executive Function. This included four measures: Two verbal fluency tests, *phonological fluency* (letter s) and *semantic fluency* (animals), each with number of words generated in 60 seconds; *Letter-Number Sequencing* is a working memory test also included in the WAIS-III. The subject is read a combination of numbers and letters and is asked to reproduce the numbers first in ascending order and then the letters in alphabetic order; *Stroop Test*, a measure of selective attention and cognitive control, requiring the subject to name the colour of ink of printed colour

words, e.g. "blue" printed in red. We used a version[1] previously used in depression and included in analyses only the time to name the colours in the incongruent part.

Factor 3. Verbal Function. This included two tests, which may also be considered as tests of semantic memory: *Familiar Faces*[20] with naming of 28 generally well-known faces, and *Boston Naming Test* with 60 objects in line drawings.

Factor 4. Verbal Learning and Memory. This consisted of two measures from *Rey Auditory Verbal Learning Test (RAVLT)*, which is a test of free recall of a list of 15 words. We included total number of words recalled in trials 1-5 and delayed recall after 30 minutes.

Additionally, UK examined all participants with the *CAMCOG,*[21] the cognitive section of The Cambridge Examination for Mental Disorders of the Elderly (CAMDEX), which is a brief neuropsychological instrument that includes measures of language processing, working memory, and declarative memory. The maximum score was 104 points.

Analyses of neuropsychological test results

All scores of the cognitive tests (except CAMCOG) were transformed to Z-scores with a mean of 0 and an SD of 1 to allow grouping of highly correlated tests into factor scores. Factors scores were computed as the average of constituent test measures and standardized so all factors had a mean of 0 and an SD of 1. Similarly, the averages of all 13 tests measures were computed and standardised to create a global summary, here termed "general cognition score". The primary outcome measure of cognitive function was change in the general cognition score, calculated as the change in the general cognition score from trial entry to after 4 weeks of intervention (T4- T0).

To estimate reliabilities of test measures, we calculated test-retest correlations in all test measures (raw scores, factor scores and general cognition score) in the placebo group.

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Test procedures

Three graduate psychology students trained and supervised by an experienced neuropsychologist (AG) conducted the neuropsychological testing. All tests were conducted in the same office, and all testing procedures were the same during the trial period. The same tester performed both the baseline and the follow up test, which was performed at the same time during the day.

Analysis of plasma escitalopram

Plasma escitalopram was measured following four weeks of intervention. The extraction and quantitation of escitalopram was carried out on an ASPEC XL combined with a high-pressure liquid chromatography (HPLC) system, both from Gilson, Villiers le Bell, France. Method validation resulted in lower and upper limits of quantitation of 10 and 3,600 nmol/l, respectively. The interassay coefficients of variation ranged from 5.5 % to 8.4%, and trueness ranged from 93.2 to 103.0% within the measurement range. Extraction recovery was 38%, and carry-over was less than 1%.

Statistical methods

Data analyses were described in a pre-established analysis plan.[11] All randomised participants were analysed, including those with missing data at the testing after four weeks of intervention. Statistical analyses were planned as ANCOVA,[22] but if the mean of the change in the difference between the results for the general cognition score and factor scores before and after the intervention did not follow, and could not be transformed into, a normal distribution, the intervention groups were compared by a non-parametric test (Mann-Whitney). Further, the outcomes were analysed as planned as the difference for the individual participants before and after the intervention, first unadjusted and then adjusted for age, sex, Hamilton depression score at

entry, and the Danish Adult Reading Test, and concentration of escitalopram in plasma, if they presented with a p-value < 0.1 in the univariate analyses.[23]

Results

Participant and non-participant characteristics

The probands (n = 466) gave us permission to contact 359 first-degree relatives, who were the potential participants in the trial. The participant flow is shown in Figure 1. A total of 80 participants were included and randomised. The clinical and demographic characteristics of the participants can be seen in Table 1.

Adherence to the intervention

One or two tablets were missed by five participants in the placebo arm and by six participants in the escitalopram arm. In the escitalopram arm two participants left the trial prior to onset of the intervention period – one man withdrew the informed consent and one female started steroid treatment due to recurrence of skin allergy. Further, data is missing for one man for the follow up test, except for CAMCOG, due to the participant's schedule problem. Full adherence to the protocol was stated by 32 participants in the placebo arm and by 33 in the escitalopram arm.

Plasma escitalopram

Blood was drawn from all 78 participants at follow up, but one test from the escitalopram group failed. The mean concentration of escitalopram was 50 nmol/l, SD 29 nmol/l, median 48 nmol/l, and range < 10 to 138 nmol/l, (n = 38) in escitalopram group. Two participants from the escitalopram group had undetectable plasma escitalopram, thus < 10 nmol/l, one of which had

stated missing the last two tablets prior to blood sampling. Plasma escitalopram was undetectable in all participants of the placebo group.

The neuropsychological tests

The test results at entry are presented in Table 2. The dataset for the neuropsychological tests was complete for 77 participants (96 %) both before (T0) and following four weeks of intervention (T4). Both groups improved considerably, presumably due to retest effects (positive values in z-scores). The change in the general cognitive function score was normally distributed (Shapiro Wilkes test). Accordingly we tested the difference between the two intervention arms with a t-test, but the difference was insignificant ($P = 0.37$, see Table 3).

In univariate analyses no statistically significant correlations were found between change in the general cognitive function score and age, sex, Hamilton depression score at entry, Danish Adult Reading Test, and plasma escitalopram. In post-hoc explorative analyses of the factors 1-4 individually, no statistically significant differences were found between the escitalopram group and the placebo group (Table 3). For the CAMCOG test, there was a statistically significant difference between the intervention groups, however, contrary to the hypothesis, treatment with escitalopram improved the CAMCOG score less than placebo (1.21 (SD: 1.92) versus 2.16 (SD: 1.98), $P = 0.04$, Table 3).

Discussion

In summary, our hypothesis that an intervention with escitalopram 10 mg would have specific beneficial effects on cognitive function in healthy first-degree relatives of patients with MDD was not supported. Thus, there was no statistically significant difference between the change in cognitive function following four weeks of intervention with escitalopram 10 mg/day compared

with matching placebo in healthy first-degree relatives of patients with MDD. The finding in the CAMCOG test is most likely a type 1 error since many outcomes were explored in this trial. Taking multiple testing into account and correcting for that would also make this finding insignificant. Consequently, improvement in cognitive function after treatment with SSRI in depressed patients seems to be related to the effects on depression symptoms.

Previous studies of the effect of SSRI on cognitive function in healthy individuals

Trials investigating the effect of SSRIs on cognitive function in healthy individuals have given inconsistent findings. In a recent review, concerning the effect of SSRIs in healthy individuals 18 trials using 39 different neuropsychological tests to investigate cognitive function were identified.[24] The findings were statistically significant differences both positive,[25-30] negative,[25;31-35] as well as neutral.[25;26;31-44] and it was concluded that the diverging findings could be a result of a number of methodological drawbacks. In general, no trial fulfilled principles of conducting and reporting randomised trials according to the Consort Statement guidelines and the majority of the trials included a mix of healthy individuals with and without a family history of affective disorders.[24] More specifically, three smaller trials have investigated the long-term effect on cognitive function of intervention of escitalopram compared to placebo in healthy individuals. Two of these studies found no significant effect of escitalopram. Wingen *et al.*,[36;39] investigated doses of escitalopram 10-20mg/day versus placebo for 15 days in a crossover design in 18 participants with an unknown family history of depression. They found no statistically significant effect on actual driving performance, psychomotor performance or visual memory performance. Paul *et al*[38] investigated escitalopram 20mg/day versus placebo for 14 days in a crossover design in 24 participants with an unknown family history of depression. They found no effect on psychomotor performance evaluated by multiple tests. In the third and most recent trial, Drueke *et al.*,[45]

administered 10 mg of escitalopram for a period of 7 days in a cross-over design to 20 healthy male participants with no family history of major mental disorder. They found differential effects of serotonergic manipulation. Thus, significant differences between escitalopram and placebo was depending on sequence of intake on attention since the test results depended on whether the test was applied for the first or the second time in relation to escitalopram and placebo. In this way, the crossover design may induce bias due to the crossover resulting in repeated multiple testing and retest effects on cognitive function. A parallel group design as used in the present trial may be superior to the cross-over design in this context.

Advantages of the AGENDA trial

The present trial is distinguished by fulfilling the criteria in the Consort Statement guidelines, and by including healthy individuals with a family history of depression only. In contrast to most studies,[24] we present information on factors that may influence outcomes such as age, gender, drug levels depression score and ethnicity. It is an advantage that the trial and the analyses were carried out as planned and the completion in the trial was very high. No participants dropped out due to adverse events. The participants were studied in a randomised clinical trial blinded in all phases including the statistical analyses and conclusion phase. The blinding was successful in relation to participants as well as researchers. Furthermore, the registered diagnoses of depression for the probands were verified by a face-to-face psychiatric research interviews by trained medical doctors. The participants were assessed and diagnosed by validated and frequently used multi-dimensional methods (e.g. SCAN interviews). Additionally, the participants were genetically homogeneous as all were ethnic Danes with European, mostly Danish, parents and grandparents. We used well-established methods for evaluations of cognitive function and we increased reliability and thus sensitivity by combining test measures. Finally, the antidepressant effect of escitalopram is generally accepted[46;47] and the participants were subjected to four weeks of

intervention thus including the interval in which clinical improvement has been reported in trials on patients with MDD.

Limitations of the trial

It is a limitation that we have not compared healthy individuals both with and without a family history of MDD. Further, a large number of women were excluded from our trial due to oral contraceptives and pregnancy, thus the trial population is characterised by an overrepresentation of men. We cannot exclude that the dosage of 10 mg escitalopram was too low although this has been suggested as the optimum dose for treatment of moderate depression.[48] Even though the participants received weekly phone calls to optimise adherence, some of the participants in the escitalopram group were found to have low plasma escitalopram concentrations. We have considered using a higher dosage, but escitalopram 20 mg daily might have given more adverse effects, possibly jeopardising blinding and adherence. The dose of escitalopram 10 mg used, resulted in well-known adverse effects as described in previous papers.[39;49]

Risk of errors

We have minimised the risk of systematic error ('bias') by using a randomised, age-and sex-stratified sample, and comparison with blinding in all phases of the trial. Also our neutral results speak against any bias. We planned to include 80 participants due to resources, feasibility and availability of the healthy first-degree relatives of patients with MDD. The AGENDA trial was planned and executed as a superiority trial and was not designed as an equivalence or non-inferiority trial.[50] Hence, we cannot exclude the possibility of overlooking a difference due to random error ('play of chance'). This issue can only be solved by further trials. Design errors may include that several participants did not reach sufficient levels of escitalopram in the blood in order to produce an effect on cognitive function. The serum escitalopram concentrations were lower than

in the study by Soegaard *et al.* [51] who found steady state plasma escitalopram concentrations of 63 ± 32 nmol/l at day 24 for escitalopram 10 mg as compared to 50 ± 29 nmol/l in our trial, though approximately 12 hours elapsed from taking the last tablet to blood sampling in our trial. Finally, we have analysed multiple outcomes thus increasing the risk of type I error for the remaining outcomes of the trial, as previously described.[11]

Generalisability

To increase the chances of detecting an effect of escitalopram versus placebo we included healthy individuals at increased risk of developing depression (i.e., with a first-degree family history of depression), as these participants seem to be to present with subtle cognitive dysfunction as previously shown in a study from our group.[3] Further, as no effect of escitalopram was found in the present trial including a group of participants at enhanced risk this finding may be generalised to healthy Caucasians without a family history of depression.

Competing interests

The trial was fully investigator initiated and controlled to secure unbiased assessment of the effect of escitalopram on healthy first-degree relatives of patients with depression.

UK, AG, PW, CG, JW and UG declare to have no competing interests. MV has been a speaker for Eli Lilly, Wyeth, Jannsen-Cilag, AstraZeneca and Pfizer. LVK has been a consultant for Bristol-Myers Squibb, Eli Lilly, Lundbeck, AstraZeneca, Servier and Jannsen-Cilag. The AGENDA trial has received non-restricted grants from non-profit and for-profit organisations.

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Authors´ contributions

Idea, planning and design: UK, MV, LVK, AG, CG, JW, UG

Conduct of the trial: UK

Statistical analyses: UK, LVK, PW, JW

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Figure 1. Flowchart for The AGENDA Trial on Cognitive Function

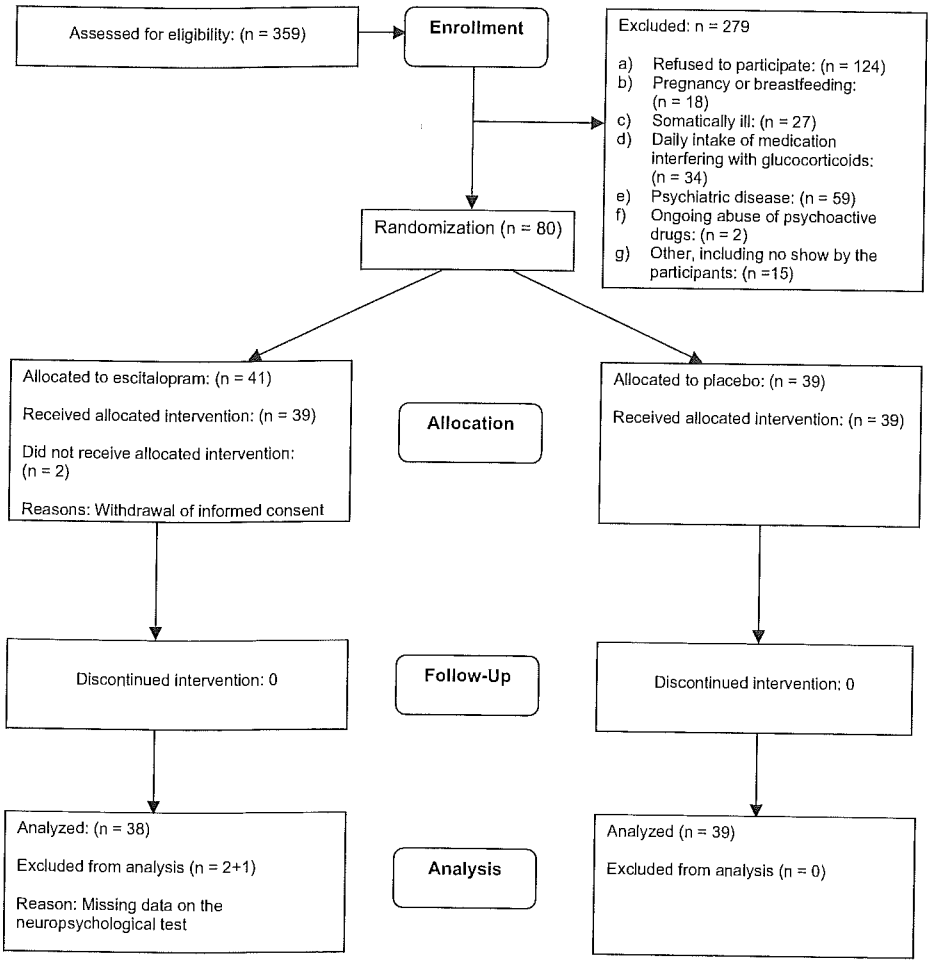

Table 1. The clinical and demographic characteristics of the participants of the AGENDA trial at entry	**Escitalopram (*n* = 41)**	**Placebo (n = 39)**	**All (*n* = 80)**
Age – yr, mean ± SD	32 ± 11	31 ± 11	32 ± 10
Women – N (%)	15 (37)	14 (36)	29 (36)
Proband was / – N (%)			
sibling	18 (44)	15 (39)	33 (41)
parent	23 (56)	24 (62)	47 (59)
Caucasian – (%)	100	100	100
Education – mean ± SD			
Years of school	11 ± 1	11 ± 1	11 ± 1
Further education score	3 ± 2	3 ± 2	3 ± 2
Employment status – N (%)			
Employed	30 (73)	26 (67)	56 (70)
Student	11 (27)	11 (28)	22 (28)
Unemployed	0 (0)	2 (5)	2 (3)
First-degree relatives of patient with a history of major depressive disorder – median (quartiles)*	1 (1;2)	1 (1;2)	1 (1;2)
Second degree relatives with a history of major depressive disorder – median (quartiles)	0 (0;1)	0 (0;1)	0 (0;1)
17-item Hamilton Depression Scale Score, – median (quartiles) (range)	1 (0;3) (0-7)	1 (0;3) (0-7)	1 (0;3) (0-7)
14-item Hamilton Anxiety Scale Score, – median (quartiles) (range)	1 (0;2) (0-9)	1 (0;2) (0-6)	1 (0;2) (0-9)
Beck Depression Inventory, 21-item, depression – median (quartiles)	2 (0;4)	2 (0;3)	2 (0;5)
Beck Depression Inventory, 14-item, anxiety – median (quartiles)	1 (0;4)	2 (0;3)	1 (0;3)
Daily medicine – N (%)**	2 (5)	4 (10)	6 (8)
Danish Adult Reading Test 45 words mean ± SD*** (range)	24.4 ± 8.4 (6-40)	25.1 ± 7.4 (8-38)	24.4 ± 7.8 (6-40)

Notes: Two participants smoked cannabis more than two months prior to the investigation. Three were previously abusing alcohol. One participant had generalized anxiety. * quartiles reported, are the 25 and 75 quartiles. **No benzodiazepines or antihistamantihistamines.*** Missing data for one participant with dyslexia.

Table 2. Neuropsychological test results at baseline for 80 first-degree relatives of patients with major depressive disorder whom participated in the AGENDA trial

Neuropsychological test	Mean	Median	SD	25 percentile	75 percentile
Symbol Digit Modalities Test	55	56	9	49	60
Trail Making A	28	27	9	21	35
Trail Making B	63	60	21	49	73
Reys complex figure, 3 min.	22	23	7	19	27
Block designs, seconds	14	12	8	10	16
Fluency for letter s	17	17	5	13	19
Fluency for animals	26	26	6	23	29
Letter number sequencing	12	12	3	11	13
Stroop (incongruence)	107	102	24	91	122
Familiar faces naming	18	20	7	12	24
Boston Naming	56	57	3	53	58
Rey Auditory Verbal Learning Test (A1A5)	50	50	8	43	56
Rey Auditory Verbal Learning Test (delay)	11	10	3	8	13
Cambridge Cognitive Examination	97	97	3	96	99

Table. 3. The distribution of changes (Δ) in results of neuropsychological test measures in first-degree relatives of the AGENDA trial following four weekes of intervention with escitalopram ($n = 38$) and placebo ($n = 39$)

Quantity	Arm (n)	Mean (SD)	Median	Minimum value	Maximum value	Inter quartile range	p	a) Normality conditions
Δ General cognition score	Escitalopram	1.17 (0.552)	1.28	-0.230	2.23	0.89	0.37	N
	Placebo	1.04 (0.693)	1.06	-0.260	2.35	0.97		
Δ Factor 1 Visuo-motor, visuo-spatial function	Escitalopram	0.544 (0.390)	0.488	-0.100	1.55	0.48	0.82	- N
	Placebo	0.423 (0.581)	0.451	-0.640	1.95	0.93		
Δ Factor 2 Executive function	Escitalopram	0.388 (0.581)	0.451	-0.640	1.95	0.93	0.27	N
	Placebo	0.229 (0.639)	0.105	-0.930	1.75	0.84		
Δ Factor 3 Verbal function	Escitalopram	0.255 (0.349)	0.255	-0.340	1.02	0.51	0.86	N
	Placebo	0.239 (0.380)	0.170	-0.590	1.27	0.51		
Δ Factor 4 Verbal learning and memory	Escitalopram	0.952 (0.655)	0.952	-0.610	2.54	0.90	0.41	(N)
	Placebo	1.05 (0.781)	1.16	-0.790	3.38	0.72		
Δ CAMCOG score	Escitalopram*	1.21 (1.92)	1	-5	5	2	0.04	(N)
	Placebo	2.16 (1.98)	2	-2	6	3		

Factor 1: Symbol Digit Modalities Test, Trail Making A and B, Reys complex figure 3 min. and Block designs.
Factor 2: Fluency for letter s, Fluency for animals, Letter number sequencing, Stroop (incongruence).
Factor 3: Familiar faces naming and Boston Naming
Factor 4: Rey Auditory Verbal Learning Test A1A5 and delay.
Δ: The difference (T4-T0) between the measurement after (T4) and before (T0) 4 weeks of intervention with escitalopram 10 mg or placebo.
a) The symbols used in this column are to be interpreted as follows N: the distributions did not differ significantly from the normal distribution (Shapiro Wilkes test), (N) they did differ but judged from the graphical displays (histograms and probability distributions) they followed normal distributions with reasonable approximation. –N: they did not follow normal distributions. In the first case a t-test was applied. In the last two cases the distributions were compared using Mann-Whitney test. * n=39 in the escitalopram group for CAMCOG score

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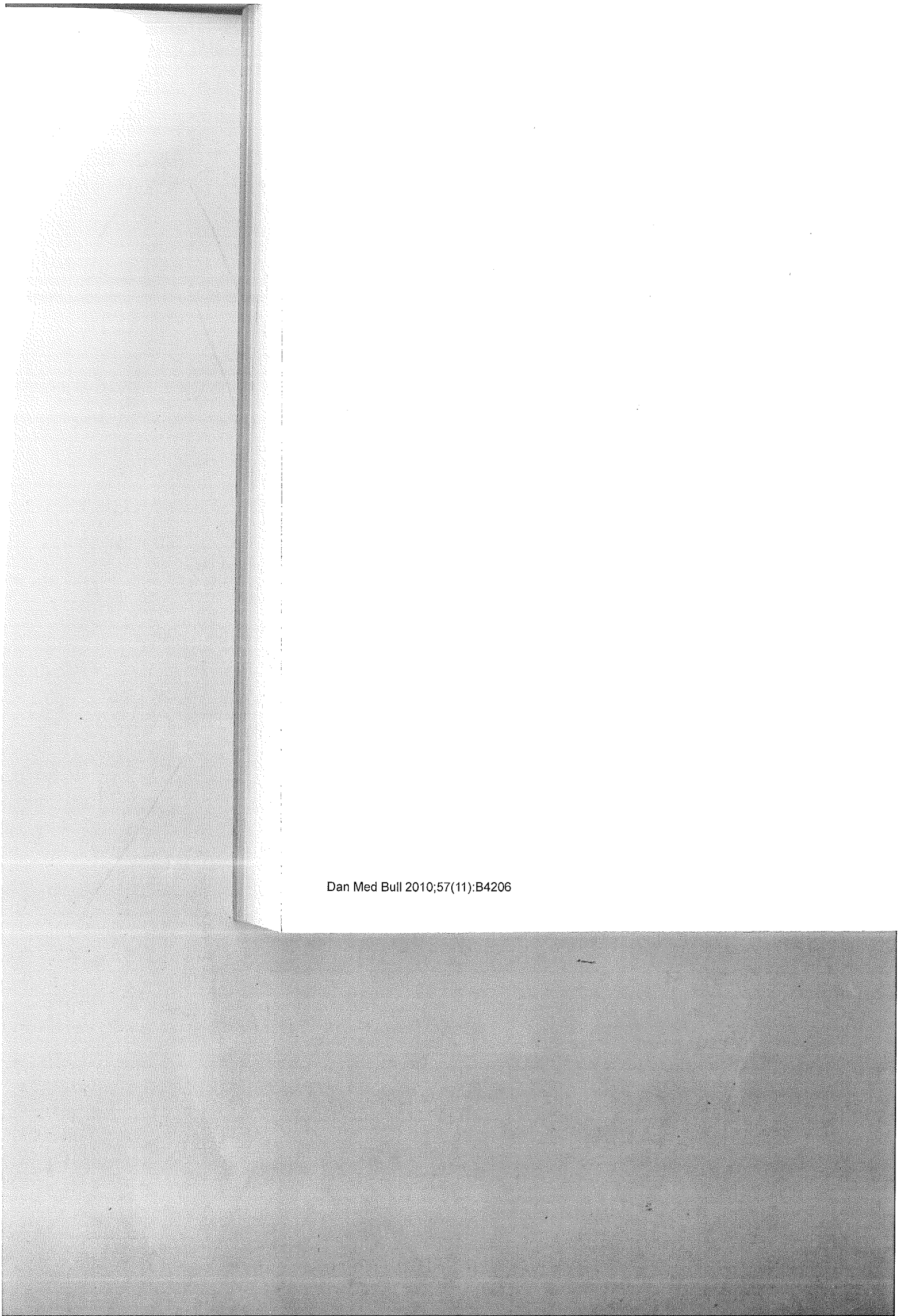

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