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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List of papers included in the thesis

Paper I
Knorr U, Vinberg M, Klose M, Feldt-Rasmussen U, Hilsted L, Gade A, Haastrup E, Paulson O, Wetterslev J, Gluud C, Gether U, and Kessing LV. Rationale and design of the participant, investigator, observer, and data-analyst-blinded randomised AGENDA trial on associations between gene-polymorphisms, endophenotypes for depression and antidepressive intervention: the effect of escitalopram versus placebo on the combined dexamethasone-corticotropin releasing hormone test and other potential endophenotypes in healthy first-degree relatives of persons with depression. Trials 2009, Aug 11; 10:66

Paper II
Knorr U, Vinberg M, Klose M, Feldt-Rasmussen U, Hilsted L, Hasselstrøm J, Gether U, Winkel P, Gluud C, Wetterslev J, and Kessing LV. Escitalopram and neuroendocrine response in healthy first-degree relatives of depressed patients – a randomised blinded trial. PLoS One. 2011; 6(6): e21224

Paper III
Knorr U, Vinberg M, Mortensen EL, Winkel P, Gluud C, Wetterslev J, Gether U and Kessing LV. A blinded randomised trial of the effect of serotonergic intervention on personality in healthy first-degree relatives of patients with depression. Accepted for publication PloS ONE (Feb 8 2012)

Paper IV
Knorr U, Vinberg M, Gade A, Winkel, P, Wetterslev J, Gluud C, Gether U, and Kessing LV.
Effect of escitalopram on cognitive function in healthy first-degree relatives of patients with depression - a blinded randomised trial. Ther Adv psychopharmacol 2011, 1:5; 133-144

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:
Decreases the plasma cortisol response in the DEX-CRH test,
Decreases self-reported scores for the personality trait neuroticism,
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 ºC. A trained bio technician and trained medical students conducted the tests under the supervision of the principal investigator.

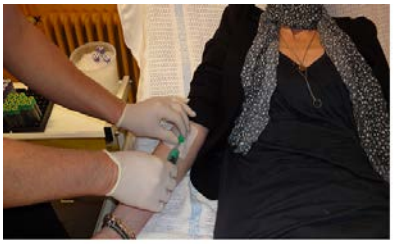

Figure 1
Conductance of the combined DEX-CRH test

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 struc-

tured 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 $\pm$ SEM) of 15,064 $\pm$ 3,947 nmol x min/l. Further, Modell et al. reported that cortisol AUC-total (mean $\pm$ SEM) in healthy individuals with no family history of MDD was 7,773 $\pm$ 1,071 nmol x min/l. A clinically relevant effect of escitalopram on the cortisol AUC-total (mean $\pm$ SEM) was thus estimated to be the difference in cortisol AUC-total (mean $\pm$ 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.

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 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.

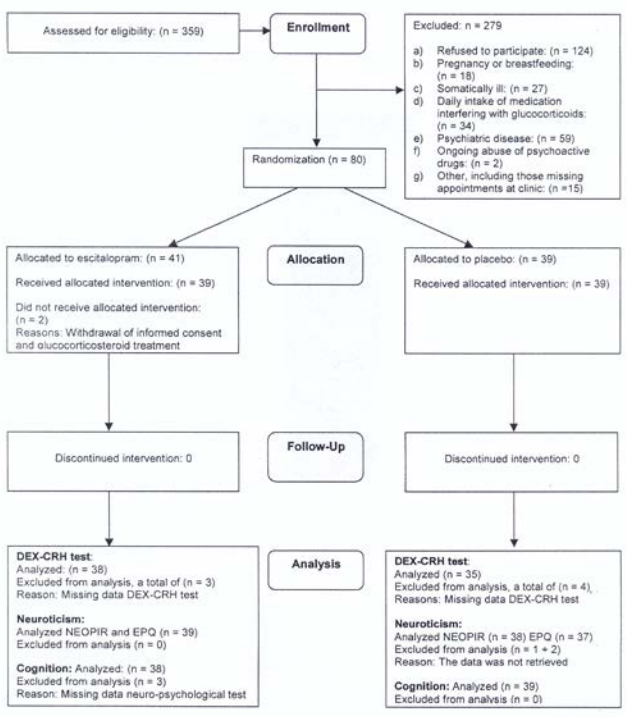

Figure 2
Flow chart for the AGENDA trial

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.

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 2

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).

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 3

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.

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 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 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 = $\ln(CorAUC_{total\cdot after}) - \ln(CorAUC_{total.before}) = \ln(CorAUC_{total.after} / CorAUC_{total.before}) = \ln(ratio)$, 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.

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 5

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.

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 6

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 7

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.

Table 8

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 re-

view 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 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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