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Article title

Does the 5-HT1A rs6295 polymorphism influence the safety and efficacy of citalopram therapy in the oldest old?

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Abstract

Major depressive disorder (MDD) in older people is a relatively common, yet hard to treat problem. In this study we aimed to establish if a single nucleotide polymorphism in the 5-HT_{1A} receptor gene (rs6295) determines antidepressant response in patients aged >80 years (the oldest old) with MDD.

Nineteen patients ≥80 years-old, with a new diagnosis of MDD were monitored for response to citalopram 20 mg daily over 4-weeks, and genotyped for the rs6295 allele. Both a frequentist and Bayesian analysis was performed on the data. Bayesian analysis answered the clinically relevant question: 'what is the probability that an older patient would enter remission after commencing SSRI treatment, conditional on their rs6295 genotype?'.

Individuals with a CC genotype showed a significant improvement in their Geriatric Depression Score (p=0.020) and cognition (p=0.035) compared to other genotypes. From a Bayesian perspective, we updated reports of antidepressant efficacy in older people with our data and calculated that the 4-week relative risk of entering remission, given a CC genotype, is 1.9 (95% HDI 0.7-3.5), compared to 0.52 (95% HDI 0.1-1.0) for the CG genotype. The sample size of n=19 is too small to draw any firm conclusions, however, the data suggest a trend indicative of a relationship between the rs6295 genotype and response to citalopram in older patients, which requires further investigation.

Keywords

Pharmacogenomics, depression, ageing, Bayesian analyis

1 Introduction

Approximately 1-4% of older adults living at home, and between 8-24% of older hospitalised inpatients are diagnosed with Major Depressive Disorder (MDD)[1], a condition characterised by either depressed mood, or diminished interest or pleasure.[2] Several factors, perhaps in combination, appear to contribute to the risk of developing the disorder in the elderly. For example, previous episodes of depression, age-related neurocognitive changes, comorbidities, and the general circumstances of old age (e.g. social isolation) may interact with each other to precipitate an episode of MDD. The symptoms of depression in later life are themselves associated with increased mortality and poor cardiovascular health.[3] This is a major concern for health services around the world as the absolute numbers of older patients with MDD is set to increase as the population ages. The need for safe, effective therapeutic interventions in this age-group is therefore of critical importance.

The first-line pharmacological treatment for depression in adults and those >65 years are the selective serotonin reuptake inhibitors (SSRIs). The rationale for using these agents is based on the serotonin hypothesis of depression first proposed by Schildkraut in 1965.[4] The model has been refined in the subsequent decades but the central tenet remains that serotoninergic signalling is disrupted in the terminals of neurones projecting from the Raphé in patients suffering MDD. A disruption to serotonergic signalling is supported by the effectiveness of SSRIs, and other antidepressants whose mode of action is through altering synaptic 5-HT levels. Nonetheless, a proportion of patients with depression, especially those who are >65 years, appear to show less response to SSRIs (around 32-44% of older patients will reach remission on an SSRI).[5,6] Several hypotheses have been proposed to explain why SSRIs lack therapeutic efficacy in some patients with MDD. These include inter-individual differences in the pharmacokinetic profile of SSRIs, and disease aetiology. However, a major focus of research over the past 15 years has been on pharmacogenetic variation in genes which code for proteins involved in 5-HT signalling.

One key target of serotonergic signalling that is of interest is the 5-HT_{1A} receptor. These receptors are coupled to inhibitory G-proteins located both post-synaptically in the corticolimbic regions of the brain, and pre-synaptically, as somatodentritic autoreceptors. Their role as autoreceptors is thought to explain the 2-3 week delay in therapeutic response to SSRIs. As SSRI therapy is commenced, the increase in synaptic 5-HT concentration stimulates 5-HT_{1A} receptors which then signal a reduction in 5-HT vesicular release, dampening any therapeutic response. After 2-3 weeks however, 5-HT_{1A} receptors desensitise, through a combination of internalisation and reduced expression, allowing the therapeutic activity of SSRIs to re-emerge. However, recent studies suggest that in a small proportion of patients, response to SSRIs may be more immediate and related to a specific pharmacogenetic trait.[7]

Because of the important role 5-HT_{1A} receptors play in 5-HT signalling, it was thought that SNPs in the gene for this receptor may determine, at least in part, an individual's susceptibility to SSRIs. For example, a functional polymorphism which increases activity or expression of the receptor may reduce treatment efficacy. Over 11 clinical trials have been conducted that have investigated the association between a common polymorphism in the promotor region of the 5-HT_{1A} receptor gene (rs6295) that results in increased receptor expression, and treatment response.[8] However, a meta-analysis which included the majority of these trials, found no overall effect of the SNP on treatment response.[9] Nevertheless, it is interesting to note that in all of the trials included in the meta-analysis, only young or middle aged adults were recruited (the mean age of participants ranged from 37 to 51 years old). This is potentially important, as recent evidence suggests that the expression of pre-synaptic 5-HT_{1A} receptors decline with age.[10] Therefore, in older people, the effect of the rs6295 SNP on SSRI treatment response may become more obvious, and therefore clinically relevant. For example, homozygote CC individuals, whose basal 5-$HT1_A$ expression is not up-regulated, will see a decline in expression during ageing, potentially leading to a more rapid SSRI response. Other genotypes may not see this increased response due their increased

burden of receptors. This hypothesis is supported by data showing that the rs6295 SNP influences response in adult patients that are treated with a partial agonist of the 5-HT_{1A} receptor (stimulating desensitisation of 5-HT_{1A} receptors) [11].

Furthermore, this decline in 5-$HT1_A$ receptor expression, coupled with the well-reported age-related decline in SERT,[12] may also lead to increased serotonergic side effects, particularly in the CC genotype. To explore this further, we conducted a clinical trial to answer the question of whether the rs6295 SNP alters response to SSRI treatment (citalopram 20 mg once daily) in hospitalised patients aged >80 years old (i.e. the oldest old), who have a first diagnosis of depression on admission to hospital.

We employed both a frequentist approach to answer this question, comparing mean and variance values statistically, and also a Bayesian approach, which is of increasing interest in medicine.[13,14] In this paper we aim to demonstrate that both analytical methods are valid approaches in pharmacogenetic trials, but that the use of Bayesian forecasting is of particular value as it 1) unambiguously addresses the relevant clinical question at hand: is a patient more likely to enter remission following 4-weeks of SSRI treatment, than not, given knowledge of their rs6295 genotype; 2) allows future studies to add their data to ours to calculate ever-more accurate relative risk values and 3) allows a meaningful analysis of data from relatively small cohorts.

2 Methods

2.1 Patients and recruitment

Study recruitment took place between January 2010 and January 2013 at Brighton and Sussex University Hospitals NHS Trust, UK. To be eligible for participation in the study, participants had to meet the following inclusion/exclusion criteria and provide written informed consent:

Inclusion criteria: ≥80 years old and admitted as an in-patient under the care of the elderly team; have a new clinical diagnosis of depression on admission, or during in-patient stay which required treatment with the local hospital formulary SSRI of choice, citalopram 20 mg once daily.

Exclusion criteria: a current prescription for an antidepressant regardless of indication; patients lacking capacity as determined by a score of ≤7/10 on a routine Abbreviated Mental Test Score (AMTS) conducted on admission .

2.2 Ethical approval

Ethics approval was granted by both NHS Research Ethics Committee South-East Coast – Brighton Sussex Research Ethics Committee Reference 09/H1107/116 and the Medicines and Healthcare products Regulatatory Agency, alongside the University of Brighton Research Ethics Committees. The study is listed on the EU Clinical Trials Register (EudraCT number 2009-016716-20). All experiemental procedures were conducted in accordance with HTA and Good Medical Practice regulations and guidelines.

2.3 Measurement of depression and clinical status

A full clinical and biochemical assessment took place at baseline, 1-week, and 4-weeks after starting SSRI treatment to determine the efficacy, tolerability and safety of citalopram therapy. Four weeks was chosen as the temporal end-point as current UK guidelines

suggest that if no repsonse is observed at this point an increase in dose or switch to an alternative medication is required. Measurements included: the Geriatric Depression Scale (GDS; 30/30 long form); Mini Mental State Examination Score (MMSE); Hunter Serotonin Toxicity Criteria (HSTC); urea and electrolytes; full blood count. Remission was defined as a GDS score of ≤11 at week 4 of the study [15].

2.4 Assessment of plasma citalopram levels

Plasma citalopram levels were determined at 4-weeks through Enzyme-Linked ImmunoSorbant Assay (Neogen®).

2.5 Assessment of platelet serotonin levels

Serotonin concentrations in platelet pellets were measured using a High Performance Liquid Chromatography (HPLC) system which consisted of a Jasco HPLC pump (Model: PU-980) and Rheodyne manual injector equipped with a 20 µl loop. A Kinetic® ODS 2.6 µm 150 mm x 4.6 mm i.d. analytical column with a guard column (Phenomenex®, Macclesfield, UK) was employed. The HPLC system was run at a flow rate of 100 µL min^{-1}. CHI630B potentiostat (CH Instruments, Austin, TX, USA) was used to control the detector voltage and record the current. A 3 mm glassy carbon electrode (flow cell, BAS) served as the working electrode and was used with a Ag|AgCl reference electrode and a stainless steel block as the auxiliary electrode. Amperometric recordings were carried out, where the working electrode was set at a potential of +950 mV vs. Ag|AgCl reference electrode. Control and data collection/processing were handled through the CHI630B software. Briefly, 500 µl of ice cold 0.1 M perchloric acid was added to the platelet pellet and samples were sonicated and vortexed for 2 minutes and then centrifuged at 14,600 x g for 10 minutes prior to chromatographic analysis. The supernatant was removed and filtered through a 0.2 µm filter and the resulting solution analysed using HPLC with electrochemical detection.

2.6 Characterisation of 5-HT_{1A} polymorphisms

DNA was extracted from 200 µl of whole blood using DNA extraction columns (DNeasy Blood and Tissue Mini-kit, Qiagen). The 5-HT_{1A} receptor gene promotor has a SNP, rs6295, at position -1019C/G which was typed by amplification using the following primers: forward 5' TGTCGTCGTTGTTCGTTTGT 3' and reverse 5' GGTGAACAGTCCTGGGTCAG 3' [16]. Amplifications were carried out in 25 µl reactions containing approximately 5 ng of DNA template and final concentrations of 15 nM 10 x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM for each deoxynucleotide (dNTP), 10 pmol forward and reverse primers, 1 unit Platinum®Taq DNA polymerase (Invitrogen). Cycling was performed in Techne TC-4000 thermal cycler employing 40 cycles (30s at 94℃, 30s at 56℃ and 60s at 72℃), with a final extension at 72℃ for 10 minutes. Sequencing (Sanger sequencing) procedures were performed by Source-Bioscience (Nottingham, England) to determine the nucleotide at position -1019.

2.7 Frequentist analysis

Chi-square tests were performed to determine the significance of any allele/genotype associations with the incidence of serotonergic side effects or the efficacy of citalopram therapy (remission, defined as a GDS score of ≤11 at week 4). General linear regression models were fitted to estimate the relative influence of demographics and genotypes on variation in response to treatment with citalopram, and the incidence of side effects. A one-way analysis of variance with Fisher's LSD post-hoc test was used to assess statistical significance of differences in the effects of citalopram according to genotype. Effect size η^2 was calculated as the sum-of-squares between groups / total sum-of-squares. To assess the statistical significance of differences in platelet [5-HT], MMSE, and GDS over time either a repeated measures one-way ANOVA, or Friedman test was used according to the distribution profile of the data. Normality of data was tested using the Shapiro-Wilk test, where we accepted the null hypothesis that the data were normally distributed if $p>0.05$. Data analysis, and graphical representation, were performed in R (R Core Team, 2016) and

Graphpad Prism v6. Statistical significance was assumed if $p<0.05$. Mean ± SEM are presented unless otherwise stated.

2.8 Bayesian forecasting

Following the method outlined in Mould et al,[18] we framed our research question in the following way: what is the conditional probability that individuals will not present in remission at week 4, i.e. a GDS of ≤11, given that they are known to have a specific genotype and that they have embarked on a course of citalopram? (The details of this approach can be found in the supplementary material).

We took the odds form of Bayes' rule: the probability of not being in remission at week 4, relative to being in remission. The appropriate prior probability distribution to employ with a likelihood that takes this form is a beta distribution [19]. This distribution has two parameters, the values of which can be drawn from previously observed data relating to the unconditional probability that an older individual will go into remission following a course of citalopram. For example, the combined remission rates in 6 studies that investigated the use of citalopram in late-life depression is calculated at around 47% (n=567).[20] In this scenario, the distribution is broadly described as symmetrically bell-shaped around a mean of 0.5 (corresponding to a probability of remission of 0.5). For comparison we also considered the the more conservative position that all possibilities for the probability of not responding to citalopram are equally likely, which is a specific case of the beta distribution and defines a non-informative uniform distribution.

To obtain the posterior distribution of the relative risk of not being in remission at week 4 relative to being in remission, conditional on the genotype, we applied Gibbs sampling in order to sample from the two distributions without having to explicitly calculate the integrals. This was performed in R (R 3.2.2, 2015) using the script employed in "OPTIMISE trial in a Bayesian framework"[21], which incorporates the scripts "openGraphSaveGraph.R" and "plotPost.R".[19]

2.9 Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

3 Results

3.1 Baseline demographics

A total of 29 patients were enrolled into the study over a 3 year period. Recruitment was more difficult than expected due to the population being acutely unwell. Ten patients missed either the final, or both follow-up visits and were therefore excluded from the analysis. The mean age of the study group was 88 ± 4 years and the mean baseline GDS for the 19 patients remaining in the study was 15/30 ± 5. The genotype frequencies of the 5-HT_{1A} receptor was found to be 7:6:6 for CC, GC and GG respectively. By comparing the observed genotype frequencies with the expected frequencies (5:9:4 for CC, GC and GG respectively), we were able to confirm that our sample population does not deviate, by any large extent, from Hardy-Weinberg equilibrium (Chi^2 = 2.554; p = 0.5263, β-1=0.59). A summary of the baseline demographics is shown in Table 1.

3.2 Response to citalopram

To measure the therapeutic effect of citalopram we compared GDS at baseline, 7 days and 4 weeks for all the patients. There was a trend towards a reduction in GDS over the 4 weeks of the study, although this was not found to be statistically significant (p = 0.11, repeated measures one-way ANOVA, n=18 [1 patient did not attend the 7 day visit and so was excluded for this analysis], Fig. 1a). A total of 8/19 patients reached remission by week 4 (i.e. a GDS score of ≤11[15]). Mean platelet [5-HT] reduced significantly over the duration of the study, indicating the pharmacological activity of citalopram on platelet 5-HT transporters, although we were only able to successfully measure concentrations in 7/19 participants (p<0.001, Friedman test; p<0.01 between baseline and 1 month, Dunn's post-hoc; n=7; Fig. 1b). Mean $[citalopram]_{plasma}$ was 0.75 ± 0.17 mg/L.

A clinical improvement in mood has previously been associated with improved cognition in older people. [22] We did show a significant correlation between change is GDS (ΔGDS) and change in MMSE (ΔMMSE) over the course of the study (R^2=0.27, p=0.02, Pearson's correlation; Fig. 1c), although we were unable to detect a significant change in mean MMSE (p=0.88, Friedman Test, n=19; Fig. 1d) over the 3 visits.

Treatment with citalopram has been associated with several side effects which could be particularly problematic to older patients. Recognised complications of citalopram therapy include hyponatraemia, and gastro-intestinal bleeding as a result of depletion of platelet 5-HT. We found a small, clinically, and statistically insignificant change in plasma Na^+ concentration over the duration of the study (mean plasma [Na^+] = 137.6 ±1.5 vs. 135.6 ±1.2 vs. 134.7 ±1.4 mmol/L at baseline, 1 week and 4 weeks respectively; (p=0.10, repeated measures one-way ANOVA, n=16, Fig. 1e). Plasma Hb concentration, which may fall due to gastrointestinal bleeding as a consequence of SSRI treatment did not change significantly over the course of the study (Fig. 1f). Another rare, but serious reaction to SSRIs is serotonin syndrome. Using the Hunter Serotonin Toxicity Criteria, one of our 19 patients was identified as positive for serotonin syndrome over the 4 week period.

3.3 Factors which determine response to citalopram

Despite the overall small decrease in mean GDS score over time, it is clear from the individual data that some patients responded well to citalopram, whilst others showed a continued deterioration in mood (Fig. 2a). To explore this further, we constructed a generalised linear model to determine whether certain baseline characteristics could predict response to citalopram (i.e. absolute change in 4-week GDS). We chose a generalised linear model due to the multinomial distribution of the data. The first iteration of the model included 4 predictor variables (5-HT_{1A} genotype, gender, age, and weight). We did not include ethnicity as a variable due to only 2 of our sample being non-white. In further iterations of the model, predictably, given the small sample size, individual variables that did not reach statistical significance were excluded until a minimum effective model was

produced [23]. The final model contained only 5-HT_{1A} genotype as a statistically significant predictor variable, explaining 32% of the deviance in GDS score (p=0.005).

3.4 The effect of $5HT_{1A}$ genotype on response to citalopram

To probe the role of 5-HT_{1A} genotype in response to citalopram, we looked at the mean change in GDS over the 4-week study according to the 3 genotypes (Fig. 2b). The mean change in GDS for the CC, GC, and GG genotypes were: -5.0 ±1.1, 1.8 ±1.3, -4.0 ±2.1 (p=0.02, One-way ANOVA with Fisher's LSD post-hoc test; n=7, 6, 6 respectively). These data show that individuals with the GC genotype demonstrate a significantly different response to citalopram at 4-weeks compared with either CC or GG (although only individuals with a CC genotype are significantly different from 0 (single sample t-test, $p<0.05$)). Interestingly, individuals with a GC genotype showed a mean increase in GDS (i.e. worsening of depression), although this is not significantly different from 0. Genotype was found to be associated with the incidence of remission at week 4 (remission/total: CC = 5/7, CG = 0/6, GG = 3/6; Chi squared test, p=0.031).

We also observed a significant reduction in absolute MMSE score in patients with a GC genotype, compared to the CC group (0.4/30 ±0.7 vs. -2.3/30 ±1.0 vs. 0.3/30 ±0.5; $p<0.05$, One-way ANOVA, Fisher's LSD post-hoc test, Fig. 2c). The role of genotype on platelet [5-HT] is inconclusive due to small numbers in each group (Fig. 2d). We found no relationship between changes in plasma Na^+ and Hb concentrations, or the presence of serotonin syndrome and genotype over the study period (data not shown).

3.5 Relative risk of not responding to Citalopram, conditional on genotype

The traditional frequentist treatment of our data presented above is indicative of an effect and, being the standard approach, is potentially of value for inter-study comparisons; however, this approach does not explicitly address the question of clinical interest. When making a decision about starting SSRI therapy, it may be more useful for a clinician to know the probability that an individual will respond poorly to citalopram, in the knowledge of their 5-HT_{1A} genotype. Bayesian inference provides a means of addressing the former question,

and also determines the probability of our model (i.e. that there is a relationship between rs6295 genotype and response to citalopram). To perform this analysis we first categorised our participants as either responders or non-responders; where *responders* are those who reached remission (GDS score ≤ 11) at week 4 and *non-responders* are those who did not. Fig. 3 shows the influence of the prior distributions (first column) on the likelihood of each genotype (first row) in arriving at the posterior relative risk of *not* responding (θ_1) to responding (θ_2). When an informative beta prior is considered, an individual that has the CG genotype is, on average, 1.79 times more likely to not remit at four weeks than to go into remission. The highest density interval (HDI) spans between 0.8 and 3; however, the probability that the CG individuals have a higher chance of not remitting than remitting is 99.3% (proportion of the posterior distribution > $\theta_1/\ \theta_2 = 1$). Fig. 4 shows the relative risk of responding (θ_1) to not responding (θ_2). In this analysis, when an informative prior is used, an individual that has the CC genotype is, on average, 1.9 times more likely to go into remission at four weeks than to not go into remission (Fig. 4). The HDI spans between 0.7 and 3.5, where the probability that the CC individuals have a higher chance of going into remission than not is 93.5%.

4 Discussion

4.1 Response to citalopram in the oldest old is related to 5-HT_{1A} receptor genotype

Our study set out to explore an important question relating to the value of 5-HT_{1A} receptor genotypes in predicting response to citalopram in the oldest old. Given the nature of our target population, recruitment was sub-optimal and our sample size is likely to be considered too small for any meaningful statistical analysis. In light of this, we wish to emphasise two key points: 1) our data are drawn from a difficult to obtain population and are likley to be of value to future studies and 2) we propose that for studies such as these, a simple Bayesian analysis is more transparent in conveying the influence of the evidence (proportional to the sample size) on any one hypothesis.

The current study has shown that for a population of depressed older patients (>80 years old) genotype explains approximately 32% of the variation in response to citalopram. We were able to exclude age, ethnicity and gender as confounders, although we acknowledge that these may have small effects that we were unable to detect with our small sample size. Other factors, which we were unable to control for may also contribute to the observed changes. Interestingly, we showed that both homozygote groups (CC and GG) displayed a mean reduction in GDS over the study period, whilst the heterozygote group's score increased, indicating a worsening of depression. The calculated effect size for genotype equates to approximately 0.37 (η^2), which is considered large. The effect size of genotype on remission rates is also large (Cramer's V=0.61), compared to similar studies conducted in younger patients (Cramer's V=0.34), which raises the intriguing possibility that the effect of genotype is more prominent in this older population. It should be noted however that whilst it appears that the results of GC group reflect an absence of response at 4 weeks, it may equally be the case that the response is delayed. Conducting the study over a longer period could resolve this question, but may prove difficult due to retention issues.

This non-linear relationship between the addition of a G allele on ΔGDS was not expected, yet this pattern was also observed in our analysis of genotype on MMSE following citalopram

treatment (the cognition of homozygotes both improved slightly over the course of the study, whilst the heterozygote group showed a decline). Indeed, post-hoc analysis revealed that the GC genotype behaves differently in response to citalopram compared to both homozygotes. Nonetheless, it should be noted that only the CC ΔGDS showed a statistically significant difference from 0 ($p<0.05$, one-sample Student's t-test). A similar observation was noted by Koto *et al*, who showed that the RS6295 heterozygote has significantly lower response and remission rates at 2 weeks compared to both homozygotes.[17]

One possible reason for this observation could be an interaction between age and phenotype. Evidence suggests that both 5-HT_{1A} receptor and SERT expression decline with increasing age.[10,12] The effect of the latter would be to increase synaptic 5-HT, further reducing 5-HT_{1A} surface expression through desensitisation (internalisation and transcriptional repression).[24] This may perhaps improve SSRI efficacy in CC individuals (as they would be susceptible to Deaf-1 repression), whilst having little effect on those carrying a G allele. Our results were consistent with our hypothesis in terms of CC individuals responding positively to citalopram, and GC individuals not so; but there is an obvious conflict in the positive response observed within our GG group. A possible explanation for this finding is summarisied in Fig. 5. Briefly, based on previous studies, we speculate that individuals with a GG genotype in the population fall into two categories: those who have a compensatory mechanism that restores 5-HT signalling, and those who do not. Those individuals who do not compensate will be more prone to depression, presenting with symptoms throughout life [25]. The idea of a compensatory mechanism in this genotype has been postulated previously and may involve a reduction in the expression of SERT, and a restoration of 5-$HT1_A$ expression to levels not dissimilar to other genotypes. It is these individuals which we believe may present late in life with depression, and, due to the down-regulation of 5-$HT1_A$ receptors may show an uncharacteristic (based on genotype) positive response to SSRI therapy. The situation may be complicated further by the fact that there are recognised gender differences in age-related changes to 5-$HT1_A$ expression, with

females showing less pre-synaptic receptor decline with age compared to men.[26] This may potentially make aged men more sensitive to SSRIs than females. Indeed, all males in our study (n=5) showed a reduction in GDS over the course of the study. At this stage however, we must caution that this interpretation is speculative, but this offers a possible hypothesis that can be tested in future studies.

Despite demonstrating a relationship between 5-HT_{1A} genotype and SSRI efficacy in old age, we did not find a role for genotype in susceptibility to common adverse reactions associated with SSRIs. Neither a reduction in plasma Na^+ or Hb concentration, or the presence of serotonin syndrome were significantly different between genotypes populations, however this does not necessarily rule out a relationship as they may take a longer period to manifest than our data collection period allowed, and it may not have been possible to detect them because of our small sample size.

4.2 Bayesian Analysis

So far in our discussions we have considered data analysed from a frequentists viewpoint and asked the statistical question: what is the probability of observing our data if there were no underlying genotype effects. For data where $p<0.05$, it is standard to consider the probability of observing our data by chance to be so small that it is more likely that there are real genotype effects. However, with an n=19, it would be prudent to exercise some caution in drawing any firm conclusions. Furthermore, when making clinical decisions about which treatment should be initiated in an individual, this type of analysis offers little help. Instead, it may be more useful to ask the following question: what is probability of observing treatment failure in an individual, given a particular genotyope – this approach is termed Bayesian forcasting and is arguably of more value to a clinician than frequentist data. Importantly for situations such as ours, the simple Bayesian approach we adopt at least offers a transparent indication of the contribution our evidence offers in support of any one hypothesis.

The Bayesian analysis we performed incorporated data from previous studies investigating response to citalopram in older patients as the prior. Limited as these data are for these

difficult to obtain samples, our Bayesian forcasting indicates that the CG and CC genotypes may influence response to citalopram in different directions and highlights the need for more studies in this area. Importantly, by adopting the methodology presented here, further studies can readily incorporate our posterior distributions as future priors, thereby fully benefitting from the strength Bayesian analysis brings to studies of this nature.

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Declaration of interest

We confirm that there are no actual or potential conflicts of financial interest with any of the authors, or the authors' respective institutions.

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Autor contributions

GS contributed to the study conception and design, wrote the manuscript, prepared Figure 1-3 and performed data analysis; AO contributed to the study conception and design, co-wrote the manuscript, generated Figures 3 and 4 and performed data analysis; RS contributed to the study conception and design, co-wrote the manuscript and performed clinical testing serotonin syndrome, cognition and depression. BP performed experimental and data analysis of platelet serotonin concentrations, prepared Figure 1b, and participated in revising the manuscript; LH performed experimental analysis of platelet serotonin concentrations, and participated in revising the manuscript; MY conceived the study, co-wrote the manuscript and performed data analysis and interpretation; JW contributed to the study conception and design, co-wrote the manuscript and performed data analysis and interpretation.

Baseline characteristics (n=19)	
Age (years)	88.16 (±3.8)
Gender (male:female)	5:14
Genotype (CC:GC:GG)	7:6:6
CrCl (mL/min)	41.01 (±30.8)
Weight (kg)	64.32 (±13.1)
Baseline MMSE	27 (±2)
Baseline GDS	15 (±5)
Hb (g/dL)	11.97 (±2.0)
WCC (x10^9/L)	9.91 (±4.7)
Plt (x10^9/L)	267.16 (±91.4)
Na (mmol/L)	138 (±6.0)
K (mmol/L)	3.9 (±0.6)
Urea (mmol/L)	9.2 (±3.6)
Ethnicity	White British 17
	Mixed White and Black African 2

Table 1. Demographic and clinical data of study recruitments. Values represent mean ± standard deviation (with the exception of gender and genotype).

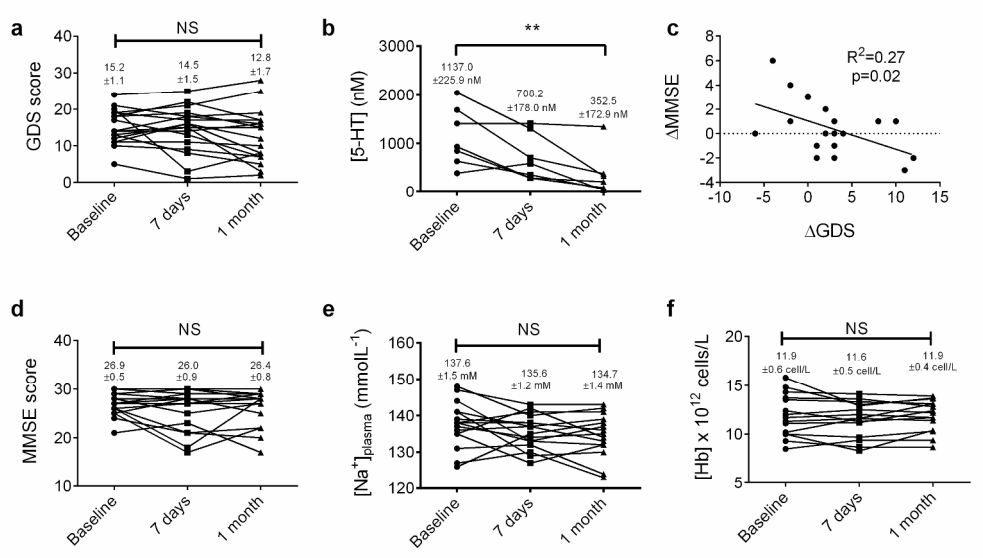
Fig. 1 Effect of citalopram on clinical and psychological measure. (a) There is small but statistically insignificant reduction in GDS over the 1 month study. (b) Platelet [5-HT] shows a significant reduction of the study period (n=7, $p<0.01$, Friedman Test). (c) There is significant correlation between the change in GDS during treatment and MMSE, although mean MMSE remains stable over the study period (d; n=19, $R^2=0.27$, p=0.02, Pearson's correlation). (e) and (f) Both plasma Na and Hb concentrations remained stable over the study period respectively

Fig. 2 The relationship between 5-HT_{1A} genotype and response to citalopram. (a) Histogram demonstrating the distribution of partipants ΔGDS scores. The appears to be 3 groups of patients: a group of responders, a group demonstrating no clinical change, and a group whose depression score declines. (b) Individuals with GC genotype were significantly different to indiviuals with a CC genotype in terms of effect on depression score (GDS; n=19, p=0.024, one-way ANOVA). (c) Individuals with GC genoype showed a significant decrease in cognition over the study period compared with CC and GG genotype (n=19, p=0.035, one-way ANOVA). (d) No statistically significant difference was found between the 3 genotypes in terms of platelet [5-HT] concentration

Fig. 3 The histograms show the posterior relative risk of not responding (θ_1), relative to responding (θ_2), conditional upon genotype using a non-informative, uniform prior (beta(1,1)), and a prior that corresponds to the literature but with a reasonable degree of uncertainty (beta(4,4))(first column). The first row gives the The likelihoods of the not-responding group for each genotype (D = data). Black horizontal bars represent 95% highest density intervals (95% chance that the true relative risk falls within this interval). Note the variable scale on the x-axis.

Fig. 4 The histograms show the posterior relative risk of responding (θ_1), relative to not responding (θ_2), conditional upon genotype using a non-informative, uniform prior (beta(1,1)), and an informative prior (beta(4,4))(first column). The first row gives the The likelihoods of the not responding group for each genotype (D =data). Black horizontal bars represent 95% highest density intervals (95% chance that the true relative risk falls within this interval). Note the variable scale on the x-axis.

Fig. 5 Proposed mechanism to explain unexpected positive response of GG individuals to citalopram. The GG genotype has been postulated to comprise two sets of individuals, those who posess a compensatory mechanism to restore 5-HT signalling, and those who do not. It might be expected that GG individuals without a compensatory mechasim will be prone to depression throughout life and require antidepressant therapy during adulthood. In this study we propose that it is GG individuals who have a compensatory mechanism that present in old age with depression that requires treatment. Due to the down-regulation of 5-HT_{1A} receptors, these individuals may be expected to respond well to SSRI therapy.



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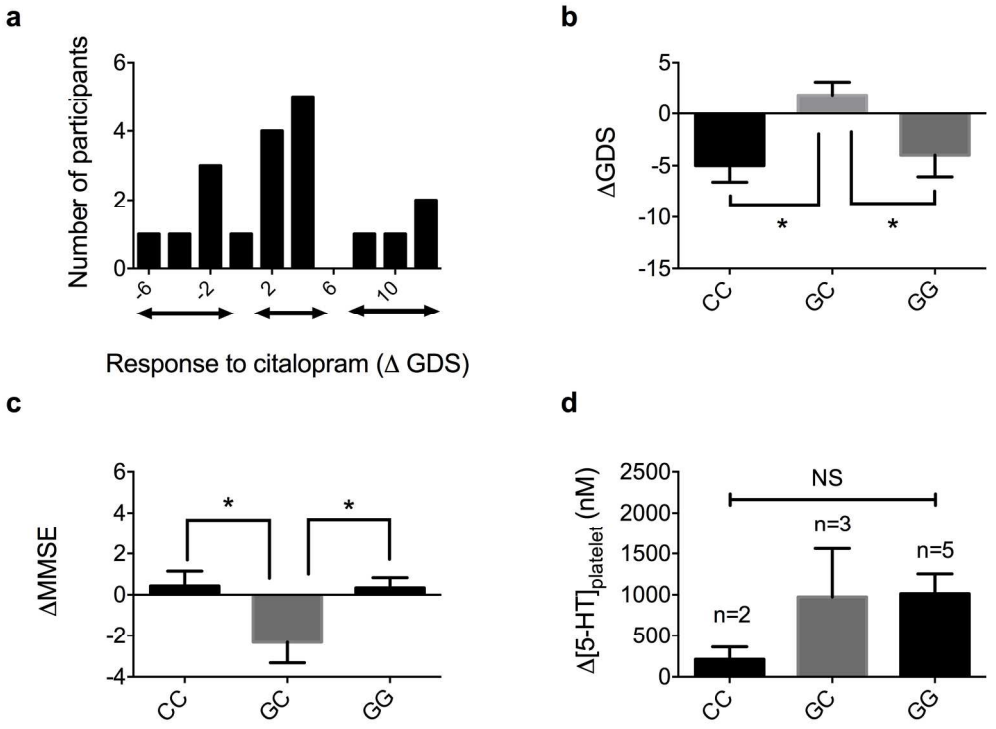

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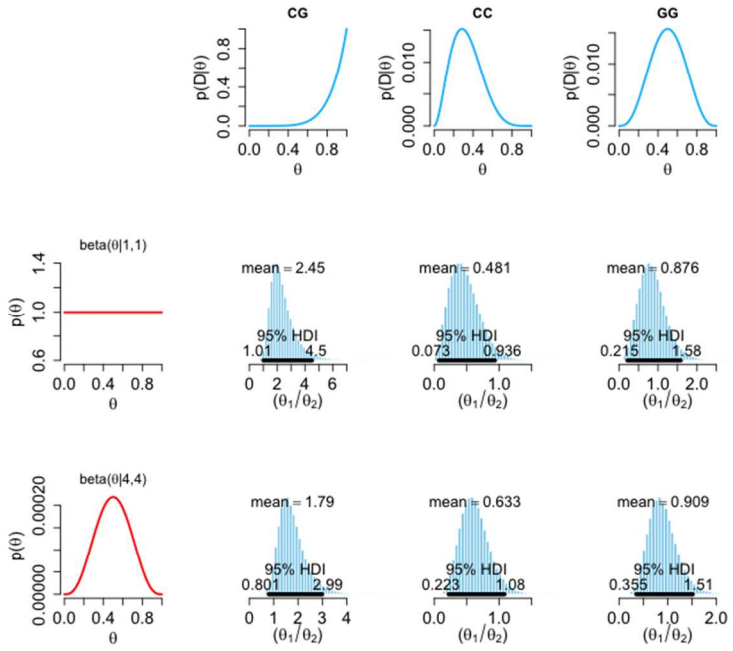

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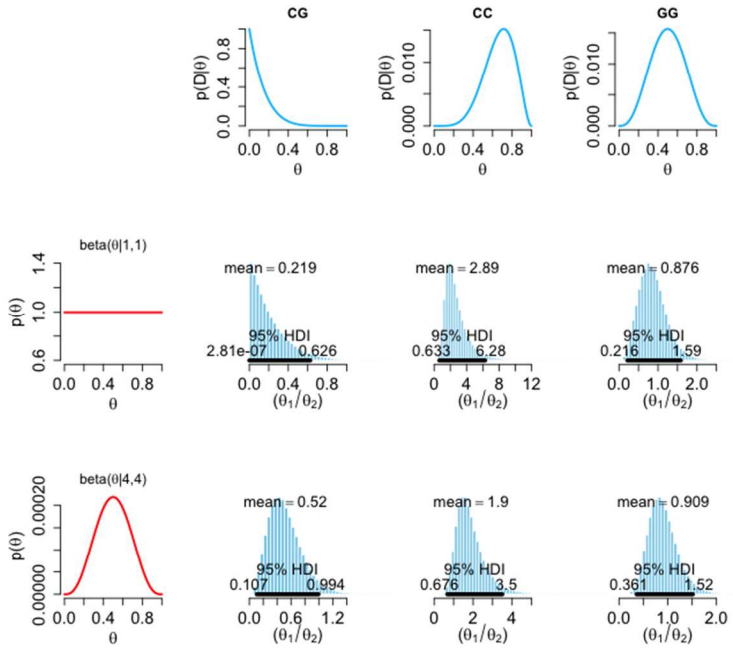

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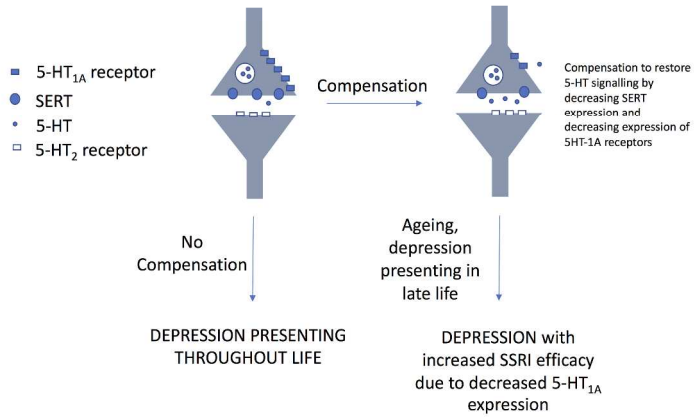

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Article type

Original article

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Article title

Does the 5-HT1A rs6295 polymorphism influence the safety and efficacy of citalopram therapy in the oldest old?

Authors

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Supplementary material

Bayesian forecasting

A major part of this study examined whether 5-HT1A receptor genotype affects the efficacy of citalopram in the oldest old. Following the method outlined in Mould et al (1), this question was framed as follows. Given that individuals have a particular genotype at the C1019G locus, what is the probability that they will or will not be in remission by week 4 of treatment with citalopram (i.e. a GDS of ≤11)? We can, for example, formulate our question as the conditional probability that individuals will not present in remission at week 4 ($\bar{\boldsymbol{R}}$), given that they are known to have a specific genotype (*G*) and that they have embarked on a course of citalopram (*C*). This conditional probability has the notation Pr($\bar{\boldsymbol{R}}$|*G*,*C*). This probability can be calculated using Bayes' rule, generally:

$$\Pr(X|\square, Z) = \frac{\Pr(Y|X,Z)\Pr(X|Z)}{\Pr(Y|Z)}$$

Giving:

$$\Pr(\bar{R}|G,C) = \frac{\Pr(G|\bar{R},C)\Pr(\bar{R}|C)}{\Pr(G|C)}$$

If we take the odds form of Bayes' rule, the probability of not being in remssion at week 4, relative to being in remission is calculated as follows:

$$\frac{\Pr(\bar{R}|G,C)}{\Pr(R|G,C)} = \frac{\Pr(G|\bar{R},C)\Pr(\bar{R}|C)}{\Pr(G|R,C)\Pr(R|C)} \quad (1)$$

Where Pr(*G*|$\bar{\boldsymbol{R}}$, $\boldsymbol{C}$) is the likelihood function for the current genotypic data and Pr($\bar{\boldsymbol{R}}$|$\boldsymbol{C}$) the prior probability of individuals not being in remission when given a course of citalopram. Because our outcome is binary (no remission/remission), we can characterize our likelihood as a binomial distribution:

$$\Pr(G|\bar{R}) \propto \bar{R}^{x} R^{n-x} \quad (2)$$

If, for example, we specify that our genotype of interest is the CG heterozygote, then *x* is the number of CG ("successes"), *n* the total sample size and *R* (= 1 - $\bar{R}$) is the probability of positively responding to citalopram (i.e. remission). The appropriate prior probability distribution to employ with a likelihood that takes this form is a beta distribution (2). This distribution has two parameters (*a* and *b*) and the probability density is defined as:

$$\Pr(\bar{R} \mid a, b) = \bar{R}^{a-1} R^{b-1} \quad (3)$$

The values of *a* and *b* that define this distribution can be drawn from previously observed data relating to the unconditional probability that an older individual will go into remission following a course of citalopram. For example, the combined remission rates in 6 studies investigating the use of citalopram in late-life depression is calculated at around 47% (n=567) (3). In this scenario, $a \approx b$. For example, where $a = b = 4$, the distribution is broadly described as symmetrically bell-shaped around a mean of 0.5 (corresponding to a probability of remission of 0.5), but where probabilities of 0.2 and 0.8 are not unreasonable. If no useful data can be obtained, we take the position that all possibilities for Pr($\bar{R}$) are equally likely, which is a specific case of the beta distribution ($a = b = 1$) and defines a non-informative uniform distribution.

To obtain the posterior distribution of relative risk (Pr($\bar{R}$|*CG, C*) / Pr(*R*|*CG, C*), eq (1)) we applied Gibbs sampling in order to sample from the two distributions (Pr($\bar{R}$|*CG, C*) and Pr(*R*|*CG, C*)), without having to explicitly calculate the integrals. This was performed in R (R 3.2.2, 2015) using the script employed in "OPTIMISE trial in a Bayesian framework" (4), which incorporates the scripts "openGraphSaveGraph.R" and "plotPost.R" (2).

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