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20 15 **Article title**

21 16 Does the 5-HT1A rs6295 polymorphism influence the safety and efficacy of citalopram
22 17 therapy in the oldest old?

23 18
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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 analysis

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 serotonergic 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.

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3 76 One key target of serotonergic signalling that is of interest is the 5-HT_{1A} receptor. These
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5 77 receptors are coupled to inhibitory G-proteins located both post-synaptically in the
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7 78 corticolimbic regions of the brain, and pre-synaptically, as somatodendritic autoreceptors.
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9 79 Their role as autoreceptors is thought to explain the 2-3 week delay in therapeutic response
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11 80 to SSRIs. As SSRI therapy is commenced, the increase in synaptic 5-HT concentration
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13 81 stimulates 5-HT_{1A} receptors which then signal a reduction in 5-HT vesicular release,
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15 82 dampening any therapeutic response. After 2-3 weeks however, 5-HT_{1A} receptors
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17 83 desensitise, through a combination of internalisation and reduced expression, allowing the
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19 84 therapeutic activity of SSRIs to re-emerge. However, recent studies suggest that in a small
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21 85 proportion of patients, response to SSRIs may be more immediate and related to a specific
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23 86 pharmacogenetic trait.⁷

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25 87 Because of the important role 5-HT_{1A} receptors play in 5-HT signalling, it was thought that
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27 88 SNPs in the gene for this receptor may determine, at least in part, an individual's
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29 89 susceptibility to SSRIs. For example, a functional polymorphism which increases activity or
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31 90 expression of the receptor may reduce treatment efficacy. Over 11 clinical trials have been
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33 91 conducted that have investigated the association between a common polymorphism in the
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35 92 promotor region of the 5-HT_{1A} receptor gene (rs6295) that results in increased receptor
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37 93 expression, and treatment response.⁸ However, a meta-analysis which included the majority
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39 94 of these trials, found no overall effect of the SNP on treatment response.⁹ Nevertheless, it is
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41 95 interesting to note that in all of the trials included in the meta-analysis, only young or middle
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43 96 aged adults were recruited (the mean age of participants ranged from 37 to 51 years old).
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45 97 This is potentially important, as recent evidence suggests that the expression of pre-synaptic
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47 98 5-HT_{1A} receptors decline with age.¹⁰ Therefore, in older people, the effect of the rs6295 SNP
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49 99 on SSRI treatment response may become more obvious, and therefore clinically relevant.
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51 100 For example, homozygote CC individuals, whose basal 5-HT_{1A} expression is not up-
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53 101 regulated, will see a decline in expression during ageing, potentially leading to a more rapid
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55 102 SSRI response. Other genotypes may not see this increased response due their increased

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3 103 burden of receptors. This hypothesis is supported by data showing that the rs6295 SNP
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5 104 influences response in adult patients that are treated with a partial agonist of the 5-HT_{1A}
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7 105 receptor (stimulating desensitisation of 5-HT_{1A} receptors)¹¹.
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10 106 Furthermore, this decline in 5-HT_{1A} receptor expression, coupled with the well-reported
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12 107 age-related decline in SERT,¹² may also lead to increased serotonergic side effects,
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14 108 particularly in the CC genotype. To explore this further, we conducted a clinical trial to
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16 109 answer the question of whether the rs6295 SNP alters response to SSRI treatment
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18 110 (citalopram 20 mg once daily) in hospitalised patients aged >80 years old (i.e. the oldest old),
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20 111 who have a first diagnosis of depression on admission to hospital.

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22 112 We employed both a frequentist approach to answer this question, comparing mean and
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24 113 variance values statistically, and also a Bayesian approach, which is of increasing interest in
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26 114 medicine.^{13,14} In this paper we aim to demonstrate that both analytical methods are valid
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28 115 approaches in pharmacogenetic trials, but that the use of Bayesian forecasting is of
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30 116 particular value as it 1) unambiguously addresses the relevant clinical question at hand: is a
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32 117 patient more likely to enter remission following 4-weeks of SSRI treatment, than not, given
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34 118 knowledge of their rs6295 genotype; 2) allows future studies to add their data to ours to
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36 119 calculate ever-more accurate relative risk values and 3) allows a meaningful analysis of data
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38 120 from relatively small cohorts.

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3 123 **2 Methods**

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6 125 2.1 Patients and recruitment

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9 127 Study recruitment took place between January 2010 and January 2013 at Brighton and
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11 128 Sussex University Hospitals NHS Trust, UK. To be eligible for participation in the study,
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13 129 participants had to meet the following inclusion/exclusion criteria and provide written
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15 130 informed consent:

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17 131 Inclusion criteria: ≥ 80 years old and admitted as an in-patient under the care of the elderly
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19 132 team; have a new clinical diagnosis of depression on admission, or during in-patient stay
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21 133 which required treatment with the local hospital formulary SSRI of choice, citalopram 20 mg
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23 134 once daily.

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26 135 Exclusion criteria: a current prescription for an antidepressant regardless of indication;
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28 136 patients lacking capacity as determined by a score of $\leq 7/10$ on a routine Abbreviated Mental
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30 137 Test Score (AMTS) conducted on admission .

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33 138 2.2 Ethical approval

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36 140 Ethics approval was granted by both NHS Research Ethics Committee South-East Coast –
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38 141 Brighton Sussex Research Ethics Committee Reference 09/H1107/116 and the Medicines
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40 142 and Healthcare products Regulatory Agency, alongside the University of Brighton
41
42 143 Research Ethics Committees. The study is listed on the EU Clinical Trials Register
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44 144 (EudraCT number 2009-016716-20). All experimental procedures were conducted in
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46 145 accordance with HTA and Good Medical Practice regulations and guidelines.

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48 146 2.3 Measurement of depression and clinical status

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51 148 A full clinical and biochemical assessment took place at baseline, 1-week, and 4-weeks after
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53 149 starting SSRI treatment to determine the efficacy, tolerability and safety of citalopram
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55 150 therapy. Four weeks was chosen as the temporal end-point as current UK guidelines

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3 151 suggest that if no response is observed at this point an increase in dose or switch to an
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5 152 alternative medication is required. Measurements included: the Geriatric Depression Scale
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7 153 (GDS; 30/30 long form); Mini Mental State Examination Score (MMSE); Hunter Serotonin
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9 154 Toxicity Criteria (HSTC); urea and electrolytes; full blood count. Remission was defined as a
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11 155 GDS score of ≤ 11 at week 4 of the study ¹⁵.

12 13 156 2.4 Assessment of plasma citalopram levels

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16 157 Plasma citalopram levels were determined at 4-weeks through Enzyme-Linked
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18 158 ImmunoSorbant Assay (Neogen[®]).

19 20 21 159 2.5 Assessment of platelet serotonin levels

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23 161 Serotonin concentrations in platelet pellets were measured using a High Performance Liquid
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25 162 Chromatography (HPLC) system which consisted of a Jasco HPLC pump (Model: PU-980)
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27 163 and Rheodyne manual injector equipped with a 20 μ l loop. A Kinetic[®] ODS 2.6 μ m 150 mm x
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29 164 4.6 mm i.d. analytical column with a guard column (Phenomenex[®], Macclesfield, UK) was
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31 165 employed. The HPLC system was run at a flow rate of 100 μ L min⁻¹. CHI630B potentiostat
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33 166 (CH Instruments, Austin, TX, USA) was used to control the detector voltage and record the
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35 167 current. A 3 mm glassy carbon electrode (flow cell, BAS) served as the working electrode
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37 168 and was used with a Ag|AgCl reference electrode and a stainless steel block as the auxiliary
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39 169 electrode. Amperometric recordings were carried out, where the working electrode was set
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41 170 at a potential of +950 mV vs. Ag|AgCl reference electrode. Control and data
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43 171 collection/processing were handled through the CHI630B software. Briefly, 500 μ l of ice cold
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45 172 0.1 M perchloric acid was added to the platelet pellet and samples were sonicated and
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47 173 vortexed for 2 minutes and then centrifuged at 14,600 x g for 10 minutes prior to
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49 174 chromatographic analysis. The supernatant was removed and filtered through a 0.2 μ m filter
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51 175 and the resulting solution analysed using HPLC with electrochemical detection.

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178 2.6 Characterisation of 5-HT_{1A} polymorphisms

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180 DNA was extracted from 200 µl of whole blood using DNA extraction columns (DNeasy
181 Blood and Tissue Mini-kit, Qiagen). The 5-HT_{1A} receptor gene promotor has a SNP, rs6295,
182 at position -1019C/G which was typed by amplification using the following primers: forward
183 5' TGTCGTCGTTGTTTCGTTTGT 3' and reverse 5' GGTGAACAGTCCTGGGTTCAG 3' ¹⁶.
184 Amplifications were carried out in 25 µl reactions containing approximately 5 ng of DNA
185 template and final concentrations of 15 nM 10 x reaction buffer, 1.5 mM MgCl₂, 0.2 mM for
186 each deoxynucleotide (dNTP), 10 pmol forward and reverse primers, 1 unit Platinum®Taq
187 DNA polymerase (Invitrogen). Cycling was performed in Techne TC-4000 thermal cycler
188 employing 40 cycles (30s at 94°C, 30s at 56°C and 60s at 72°C), with a final extension at
189 72°C for 10 minutes. Sequencing (Sanger sequencing) procedures were performed by
190 Source-Bioscience (Nottingham, England) to determine the nucleotide at position -1019.

191 2.7 Frequentist analysis

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

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3 206 Graphpad Prism v6. Statistical significance was assumed if $p < 0.05$. Mean \pm SEM are
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5 207 presented unless otherwise stated.

6 7 208 2.8 Bayesian forecasting

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9 209 Following the method outlined in Mould et al,¹⁸ we framed our research question in the
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11 210 following way: what is the conditional probability that individuals will not present in remission
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13 211 at week 4, i.e. a GDS of ≤ 11 , given that they are known to have a specific genotype and that
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15 212 they have embarked on a course of citalopram? (The details of this approach can be found
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17 213 in the supplementary material).

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20 214 We took the odds form of Bayes' rule: the probability of not being in remission at week 4,
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22 215 relative to being in remission. The appropriate prior probability distribution to employ with a
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24 216 likelihood that takes this form is a beta distribution¹⁹. This distribution has two parameters,
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26 217 the values of which can be drawn from previously observed data relating to the unconditional
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28 218 probability that an older individual will go into remission following a course of citalopram. For
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30 219 example, the combined remission rates in 6 studies that investigated the use of citalopram in
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32 220 late-life depression is calculated at around 47% ($n=567$).²⁰ In this scenario, the distribution
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34 221 is broadly described as symmetrically bell-shaped around a mean of 0.5 (corresponding to a
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36 222 probability of remission of 0.5). For comparison we also considered the the more
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38 223 conservative position that all possibilities for the probability of not responding to citalopram
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40 224 are equally likely, which is a specific case of the beta distribution and defines a non-
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42 225 informative uniform distribution.

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44 226 To obtain the posterior distribution of the relative risk of not being in remission at week 4
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46 227 relative to being in remission, conditional on the genotype, we applied Gibbs sampling in
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48 228 order to sample from the two distributions without having to explicitly calculate the integrals.
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50 229 This was performed in R (R 3.2.2, 2015) using the script employed in "OPTIMISE trial in a
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52 230 Bayesian framework"²¹, which incorporates the scripts "openGraphSaveGraph.R" and
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54 231 "plotPost.R".¹⁹

232 2.9 Data availability

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

235 **3 Results**

236 3.1 Baseline demographics

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

247 3.2 Response to citalopram

248 To measure the therapeutic effect of citalopram we compared GDS at baseline, 7 days and
249 4 weeks for all the patients. There was a trend towards a reduction in GDS over the 4
250 weeks of the study, although this was not found to be statistically significant ($p = 0.11$,
251 repeated measures one-way ANOVA, $n=18$ [1 patient did not attend the 7 day visit and so
252 was excluded for this analysis], Fig. 1a). A total of 8/19 patients reached remission by week
253 4 (i.e. a GDS score of ≤ 11 ¹⁵). Mean platelet [5-HT] reduced significantly over the duration of
254 the study, indicating the pharmacological activity of citalopram on platelet 5-HT transporters,
255 although we were only able to successfully measure concentrations in 7/19 participants
256 ($p < 0.001$, Friedman test; $p < 0.01$ between baseline and 1 month, Dunn's post-hoc; $n=7$; Fig.
257 1b). Mean [citalopram]_{plasma} was 0.75 ± 0.17 mg/L.

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3 258 A clinical improvement in mood has previously been associated with improved cognition in
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5 259 older people.²² We did show a significant correlation between change in GDS (Δ GDS) and
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7 260 change in MMSE (Δ MMSE) over the course of the study ($R^2=0.27$, $p=0.02$, Pearson's
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9 261 correlation; Fig. 1c), although we were unable to detect a significant change in mean MMSE
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11 262 ($p=0.88$, Friedman Test, $n=19$; Fig. 1d) over the 3 visits.

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13 263 Treatment with citalopram has been associated with several side effects which could be
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15 264 particularly problematic to older patients. Recognised complications of citalopram therapy
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17 265 include hyponatraemia, and gastro-intestinal bleeding as a result of depletion of platelet 5-
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19 266 HT. We found a small, clinically, and statistically insignificant change in plasma Na^+
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21 267 concentration over the duration of the study (mean plasma $[\text{Na}^+] = 137.6 \pm 1.5$ vs. 135.6 ± 1.2
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23 268 vs. 134.7 ± 1.4 mmol/L at baseline, 1 week and 4 weeks respectively; ($p=0.10$, repeated
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25 269 measures one-way ANOVA, $n=16$, Fig. 1e). Plasma Hb concentration, which may fall due to
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27 270 gastrointestinal bleeding as a consequence of SSRI treatment did not change significantly
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29 271 over the course of the study (Fig. 1f). Another rare, but serious reaction to SSRIs is
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31 272 serotonin syndrome. Using the Hunter Serotonin Toxicity Criteria, one of our 19 patients
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33 273 was identified as positive for serotonin syndrome over the 4 week period.

34 35 36 274 3.3 Factors which determine response to citalopram

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38 275 Despite the overall small decrease in mean GDS score over time, it is clear from the
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40 276 individual data that some patients responded well to citalopram, whilst others showed a
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42 277 continued deterioration in mood (Fig. 2a). To explore this further, we constructed a
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44 278 generalised linear model to determine whether certain baseline characteristics could predict
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46 279 response to citalopram (i.e. absolute change in 4-week GDS). We chose a generalised
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48 280 linear model due to the multinomial distribution of the data. The first iteration of the model
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50 281 included 4 predictor variables (5-HT_{1A} genotype, gender, age, and weight). We did not
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52 282 include ethnicity as a variable due to only 2 of our sample being non-white. In further
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54 283 iterations of the model, predictably, given the small sample size, individual variables that did
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56 284 not reach statistical significance were excluded until a minimum effective model was

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3 285 produced²³. The final model contained only 5-HT_{1A} genotype as a statistically significant
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5 286 predictor variable, explaining 32% of the deviance in GDS score ($p=0.005$).

6 7 287 3.4 The effect of 5HT_{1A} genotype on response to citalopram

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9 288 To probe the role of 5-HT_{1A} genotype in response to citalopram, we looked at the mean
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11 289 change in GDS over the 4-week study according to the 3 genotypes (Fig. 2b). The mean
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13 290 change in GDS for the CC, GC, and GG genotypes were: -5.0 ± 1.1 , 1.8 ± 1.3 , -4.0 ± 2.1
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15 291 ($p=0.02$, One-way ANOVA with Fisher's LSD post-hoc test; $n=7, 6, 6$ respectively). These
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17 292 data show that individuals with the GC genotype demonstrate a significantly different
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19 293 response to citalopram at 4-weeks compared with either CC or GG (although only individuals
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21 294 with a CC genotype are significantly different from 0 (single sample t-test, $p<0.05$)).
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23 295 Interestingly, individuals with a GC genotype showed a mean increase in GDS (i.e.
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25 296 worsening of depression), although this is not significantly different from 0. Genotype was
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27 297 found to be associated with the incidence of remission at week 4 (remission/total: CC = 5/7,
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29 298 CG = 0/6, GG = 3/6; Chi squared test, $p=0.031$).

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32 299 We also observed a significant reduction in absolute MMSE score in patients with a GC
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34 300 genotype, compared to the CC group ($0.4/30 \pm 0.7$ vs. $-2.3/30 \pm 1.0$ vs. $0.3/30 \pm 0.5$; $p<0.05$,
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36 301 One-way ANOVA, Fisher's LSD post-hoc test, Fig. 2c). The role of genotype on platelet [5-
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38 302 HT] is inconclusive due to small numbers in each group (Fig. 2d). We found no relationship
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40 303 between changes in plasma Na⁺ and Hb concentrations, or the presence of serotonin
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42 304 syndrome and genotype over the study period (data not shown).

43 44 305 3.5 Relative risk of not responding to Citalopram, conditional on genotype

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46 306 The traditional frequentist treatment of our data presented above is indicative of an effect
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48 307 and, being the standard approach, is potentially of value for inter-study comparisons;
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50 308 however, this approach does not explicitly address the question of clinical interest. When
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52 309 making a decision about starting SSRI therapy, it may be more useful for a clinician to know
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54 310 the probability that an individual will respond poorly to citalopram, in the knowledge of their
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56 311 5-HT_{1A} genotype. Bayesian inference provides a means of addressing the former question,

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3 312 and also determines the probability of our model (i.e. that there is a relationship between
4 313 rs6295 genotype and response to citalopram). To perform this analysis we first categorised
5 314 our participants as either responders or non-responders; where *responders* are those who
6 315 reached remission (GDS score ≤ 11) at week 4 and *non-responders* are those who did not.
7
8 316 Fig. 3 shows the influence of the prior distributions (first column) on the likelihood of each
9 317 genotype (first row) in arriving at the posterior relative risk of *not* responding (θ_1) to
10 318 responding (θ_2). When an informative beta prior is considered, an individual that has the CG
11 319 genotype is, on average, 1.79 times more likely to not remit at four weeks than to go into
12 320 remission. The highest density interval (HDI) spans between 0.8 and 3; however, the
13 321 probability that the CG individuals have a higher chance of not remitting than remitting is
14 322 99.3% (proportion of the posterior distribution $> \theta_1 / \theta_2 = 1$). Fig. 4 shows the relative risk of
15 323 responding (θ_1) to not responding (θ_2). In this analysis, when an informative prior is used, an
16 324 individual that has the CC genotype is, on average, 1.9 times more likely to go into remission
17 325 at four weeks than to not go into remission (Fig. 4). The HDI spans between 0.7 and 3.5,
18 326 where the probability that the CC individuals have a higher chance of going into remission
19 327 than not is 93.5%.

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3 332 4 Discussion

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5 333 4.1 Response to citalopram in the oldest old is related to 5-HT_{1A} receptor genotype

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7 334 Our study set out to explore an important question relating to the value of 5-HT_{1A} receptor
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9 335 genotypes in predicting response to citalopram in the oldest old. Given the nature of our
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11 336 target population, recruitment was sub-optimal and our sample size is likely to be considered
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13 337 too small for any meaningful statistical analysis. In light of this, we wish to emphasise two
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15 338 key points: 1) our data are drawn from a difficult to obtain population and are likely to be of
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17 339 value to future studies and 2) we propose that for studies such as these, a simple Bayesian
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19 340 analysis is more transparent in conveying the influence of the evidence (proportional to the
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21 341 sample size) on any one hypothesis.

22
23 342 The current study has shown that for a population of depressed older patients (>80 years old)
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25 343 genotype explains approximately 32% of the variation in response to citalopram. We were
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27 344 able to exclude age, ethnicity and gender as confounders, although we acknowledge that
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29 345 these may have small effects that we were unable to detect with our small sample size.
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31 346 Other factors, which we were unable to control for may also contribute to the observed
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33 347 changes. Interestingly, we showed that both homozygote groups (CC and GG) displayed a
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35 348 mean reduction in GDS over the study period, whilst the heterozygote group's score
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37 349 increased, indicating a worsening of depression. The calculated effect size for genotype
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39 350 equates to approximately 0.37 (η^2), which is considered large. The effect size of genotype
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41 351 on remission rates is also large (Cramer's $V=0.61$), compared to similar studies conducted in
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43 352 younger patients (Cramer's $V=0.34$), which raises the intriguing possibility that the effect of
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45 353 genotype is more prominent in this older population. It should be noted however that whilst it
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47 354 appears that the results of GC group reflect an absence of response at 4 weeks, it may
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49 355 equally be the case that the response is delayed. Conducting the study over a longer period
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51 356 could resolve this question, but may prove difficult due to retention issues.

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54 357 This non-linear relationship between the addition of a G allele on Δ GDS was not expected,
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56 358 yet this pattern was also observed in our analysis of genotype on MMSE following citalopram

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3 359 treatment (the cognition of homozygotes both improved slightly over the course of the study,
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5 360 whilst the heterozygote group showed a decline). Indeed, post-hoc analysis revealed that the
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7 361 GC genotype behaves differently in response to citalopram compared to both homozygotes.
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9 362 Nonetheless, it should be noted that only the CC Δ GDS showed a statistically significant
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11 363 difference from 0 ($p < 0.05$, one-sample Student's t-test). A similar observation was noted by
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13 364 Koto *et al*, who showed that the RS6295 heterozygote has significantly lower response and
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15 365 remission rates at 2 weeks compared to both homozygotes.¹⁷

16
17 366 One possible reason for this observation could be an interaction between age and
18
19 367 phenotype. Evidence suggests that both 5-HT_{1A} receptor and SERT expression decline with
20
21 368 increasing age.^{10,12} The effect of the latter would be to increase synaptic 5-HT, further
22
23 369 reducing 5-HT_{1A} surface expression through desensitisation (internalisation and
24
25 370 transcriptional repression).²⁴ This may perhaps improve SSRI efficacy in CC individuals (as
26
27 371 they would be susceptible to Deaf-1 repression), whilst having little effect on those carrying a
28
29 372 G allele. Our results were consistent with our hypothesis in terms of CC individuals
30
31 373 responding positively to citalopram, and GC individuals not so; but there is an obvious
32
33 374 conflict in the positive response observed within our GG group. A possible explanation for
34
35 375 this finding is summarised in Fig. 5. Briefly, based on previous studies, we speculate that
36
37 376 individuals with a GG genotype in the population fall into two categories: those who have a
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39 377 compensatory mechanism that restores 5-HT signalling, and those who do not. Those
40
41 378 individuals who do not compensate will be more prone to depression, presenting with
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43 379 symptoms throughout life²⁵. The idea of a compensatory mechanism in this genotype has
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45 380 been postulated previously and may involve a reduction in the expression of SERT, and a
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47 381 restoration of 5-HT_{1A} expression to levels not dissimilar to other genotypes. It is these
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49 382 individuals which we believe may present late in life with depression, and, due to the down-
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51 383 regulation of 5-HT_{1A} receptors may show an uncharacteristic (based on genotype) positive
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53 384 response to SSRI therapy. The situation may be complicated further by the fact that there
54
55 385 are recognised gender differences in age-related changes to 5-HT_{1A} expression, with
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3 386 females showing less pre-synaptic receptor decline with age compared to men.²⁶ This may
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5 387 potentially make aged men more sensitive to SSRIs than females. Indeed, all males in our
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7 388 study (n=5) showed a reduction in GDS over the course of the study. At this stage however,
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9 389 we must caution that this interpretation is speculative, but this offers a possible hypothesis
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11 390 that can be tested in future studies.

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13 391 Despite demonstrating a relationship between 5-HT_{1A} genotype and SSRI efficacy in old age,
14
15 392 we did not find a role for genotype in susceptibility to common adverse reactions associated
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17 393 with SSRIs. Neither a reduction in plasma Na⁺ or Hb concentration, or the presence of
18
19 394 serotonin syndrome were significantly different between genotypes populations, however
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21 395 this does not necessarily rule out a relationship as they may take a longer period to manifest
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23 396 than our data collection period allowed, and it may not have been possible to detect them
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25 397 because of our small sample size.

26 27 28 398 4.2 Bayesian Analysis

29
30 399 So far in our discussions we have considered data analysed from a frequentists viewpoint
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32 400 and asked the statistical question: what is the probability of observing our data if there were
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34 401 no underlying genotype effects. For data where $p < 0.05$, it is standard to consider the
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36 402 probability of observing our data by chance to be so small that it is more likely that there are
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38 403 real genotype effects. However, with an n=19, it would be prudent to exercise some caution
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40 404 in drawing any firm conclusions. Furthermore, when making clinical decisions about which
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42 405 treatment should be initiated in an individual, this type of analysis offers little help. Instead, it
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44 406 may be more useful to ask the following question: what is probability of observing treatment
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46 407 failure in an individual, given a particular genotype – this approach is termed Bayesian
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48 408 forecasting and is arguably of more value to a clinician than frequentist data. Importantly for
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50 409 situations such as ours, the simple Bayesian approach we adopt at least offers a transparent
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52 410 indication of the contribution our evidence offers in support of any one hypothesis.

53
54 411 The Bayesian analysis we performed incorporated data from previous studies investigating
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56 412 response to citalopram in older patients as the prior. Limited as these data are for these

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3 413 difficult to obtain samples, our Bayesian forecasting indicates that the CG and CC genotypes
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5 414 may influence response to citalopram in different directions and highlights the need for more
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7 415 studies in this area. Importantly, by adopting the methodology presented here, further
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9 416 studies can readily incorporate our posterior distributions as future priors, thereby fully
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11 417 benefitting from the strength Bayesian analysis brings to studies of this nature.

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For Peer Review

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16
17 426 competing financial interests in relation the work described in this paper.
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20 427

21
22 428 **Declaration of interest**
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24
25 429 We confirm that there are no actual or potential conflicts of financial interest with any of the
26
27 430 authors, or the authors' respective institutions.
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30 **Author contributions**

31
32 505 GS contributed to the study conception and design, wrote the manuscript, prepared Figure
33 506 1-3 and performed data analysis; AO contributed to the study conception and design, co-
34 507 wrote the manuscript, generated Figures 3 and 4 and performed data analysis; RS
35 508 contributed to the study conception and design, co-wrote the manuscript and performed
36 509 clinical testing serotonin syndrome, cognition and depression. BP performed experimental
37 510 and data analysis of platelet serotonin concentrations, prepared Figure 1b, and participated
38 511 in revising the manuscript; LH performed experimental analysis of platelet serotonin
39 512 concentrations, and participated in revising the manuscript; MY conceived the study, co-
40 513 wrote the manuscript and performed data analysis and interpretation; JW contributed to the
41 514 study conception and design, co-wrote the manuscript and performed data analysis and
42 515 interpretation.
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Baseline characteristics (n=19)	
Age (years)	88.16 (\pm 3.8)
Gender (male:female)	5:14
Genotype (CC:GC:GG)	7:6:6
CrCl (mL/min)	41.01 (\pm 30.8)
Weight (kg)	64.32 (\pm 13.1)
Baseline MMSE	27 (\pm 2)
Baseline GDS	15 (\pm 5)
Hb (g/dL)	11.97 (\pm 2.0)
WCC ($\times 10^9/L$)	9.91 (\pm 4.7)
Plt ($\times 10^9/L$)	267.16 (\pm 91.4)
Na (mmol/L)	138 (\pm 6.0)
K (mmol/L)	3.9 (\pm 0.6)
Urea (mmol/L)	9.2 (\pm 3.6)
Ethnicity	White British 17 Mixed White and Black African 2

517

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

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3 521 Fig. 1 Effect of citalopram on clinical and psychological measure. (a) There is small but
4
5 522 statistically insignificant reduction in GDS over the 1 month study. (b) Platelet [5-HT] shows
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7 523 a significant reduction of the study period ($n=7$, $p<0.01$, Friedman Test). (c) There is
8
9 524 significant correlation between the change in GDS during treatment and MMSE, although
10
11 525 mean MMSE remains stable over the study period (d; $n=19$, $R^2=0.27$, $p=0.02$, Pearson's
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13 526 correlation). (e) and (f) Both plasma Na and Hb concentrations remained stable over the
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15 527 study period respectively

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20 529 Fig. 2 The relationship between 5-HT_{1A} genotype and response to citalopram. (a) Histogram
21
22 530 demonstrating the distribution of participants Δ GDS scores. The appears to be 3 groups of
23
24 531 patients: a group of responders, a group demonstrating no clinical change, and a group
25
26 532 whose depression score declines. (b) Individuals with GC genotype were significantly
27
28 533 different to individuals with a CC genotype in terms of effect on depression score (GDS; $n=19$,
29
30 534 $p=0.024$, one-way ANOVA). (c) Individuals with GC genotype showed a significant decrease
31
32 535 in cognition over the study period compared with CC and GG genotype ($n=19$, $p=0.035$, one-
33
34 536 way ANOVA). (d) No statistically significant difference was found between the 3 genotypes
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36 537 in terms of platelet [5-HT] concentration

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41 539 Fig. 3 The histograms show the posterior relative risk of not responding (θ_1), relative to
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43 540 responding (θ_2), conditional upon genotype using a non-informative, uniform prior ($\text{beta}(1,1)$),
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45 541 and a prior that corresponds to the literature but with a reasonable degree of uncertainty
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47 542 ($\text{beta}(4,4)$)(first column). The first row gives the The likelihoods of the not-responding group
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49 543 for each genotype ($D = \text{data}$). Black horizontal bars represent 95% highest density intervals
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51 544 (95% chance that the true relative risk falls within this interval). Note the variable scale on
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53 545 the x-axis.

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3 547 Fig. 4 The histograms show the posterior relative risk of responding (θ_1), relative to not
4 548 responding (θ_2), conditional upon genotype using a non-informative, uniform prior ($\text{beta}(1,1)$),
5 549 and an informative prior ($\text{beta}(4,4)$)(first column). The first row gives the The likelihoods of
6
7 550 the not responding group for each genotype (D =data). Black horizontal bars represent 95%
8
9 551 highest density intervals (95% chance that the true relative risk falls within this interval). Note
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11 552 the variable scale on the x-axis.
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18 554 Fig. 5 Proposed mechanism to explain unexpected positive response of GG individuals to
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20 555 citalopram. The GG genotype has been postulated to comprise two sets of individuals,
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22 556 those who possess a compensatory mechanism to restore 5-HT signalling, and those who do
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24 557 not. It might be expected that GG individuals without a compensatory mechanism will be
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26 558 prone to depression throughout life and require antidepressant therapy during adulthood. In
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28 559 this study we propose that it is GG individuals who have a compensatory mechanism that
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30 560 present in old age with depression that requires treatment. Due to the down-regulation of 5-
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32 561 HT_{1A} receptors, these individuals may be expected to respond well to SSRI therapy.
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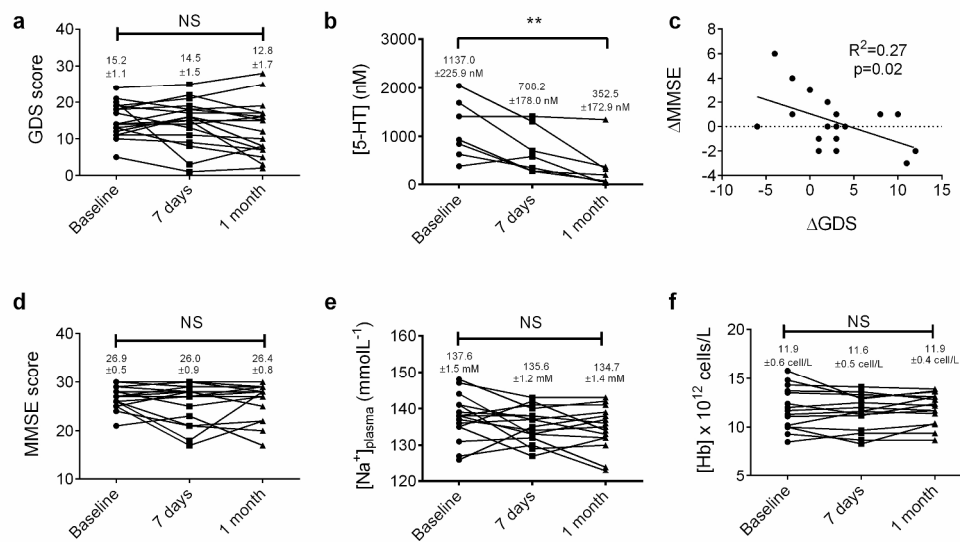


Fig. 1 Effect of citalopram on clinical and psychological measure. (a) There is small but statistically insignificant reduction in GDS over the 1 month study. (b) Platelet [5-HT] shows a significant reduction of the study period (n=7, p<0.01, Friedman Test). (c) There is significant correlation between the change in GDS during treatment and MMSE, although mean MMSE remains stable over the study period (d; n=19, R²=0.27, p=0.02, Pearson's correlation). (e) and (f) Both plasma Na and Hb concentrations remained stable over the study period respectively

298x172mm (300 x 300 DPI)

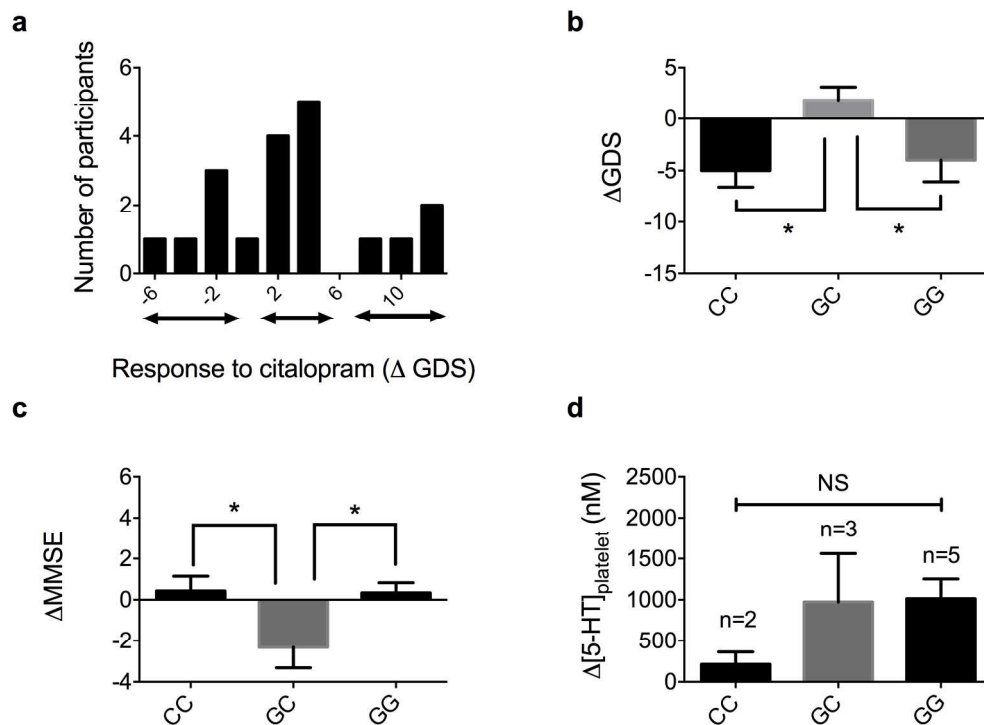


Fig. 2 The relationship between 5-HT_{1A} genotype and response to citalopram. (a) Histogram demonstrating the distribution of participants GDS scores. The appears to be 3 groups of patients: a group of responders, a group demonstrating no clinical change, and a group whose depression score declines. (b) Individuals with GC genotype were significantly different to individuals with a CC genotype in terms of effect on depression score (GDS; $n=19$, $p=0.024$, one-way ANOVA). (c) Individuals with GC genotype 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

199x149mm (300 x 300 DPI)

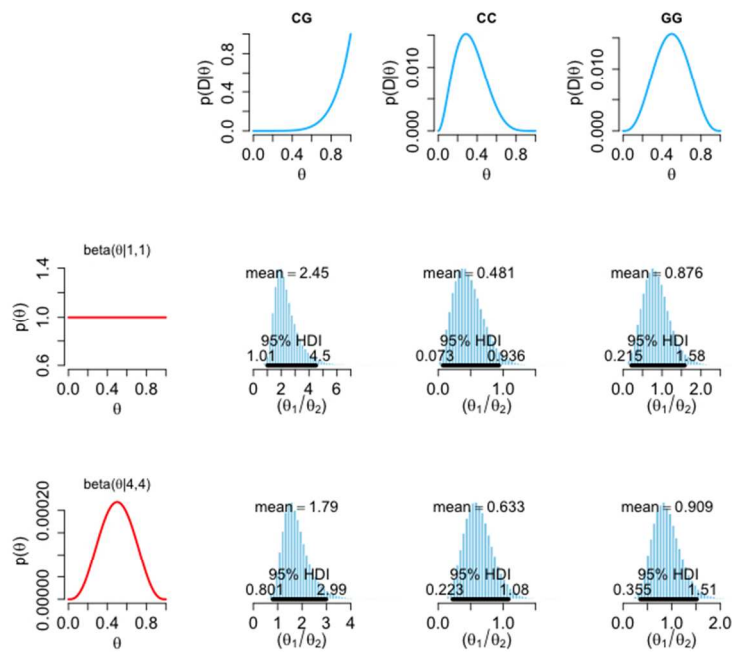


Fig. 3 The histograms show the posterior relative risk of not responding (θ_1), relative to responding (θ_2), conditional upon genotype using a non-informative, uniform prior ($\text{beta}(1,1)$), and a prior that corresponds to the literature but with a reasonable degree of uncertainty ($\text{beta}(4,4)$)(first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

254x366mm (72 x 72 DPI)

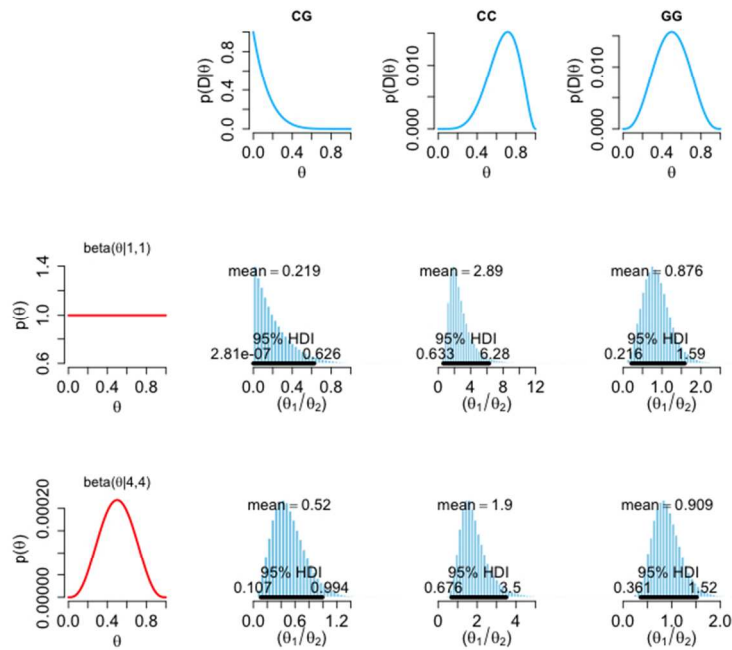


Fig. 4 The histograms show the posterior relative risk of responding (θ_1), relative to not responding (θ_2), conditional upon genotype using a non-informative, uniform prior ($\text{beta}(1,1)$), and an informative prior ($\text{beta}(4,4)$)(first column). The first row gives the The likelihoods of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

254x366mm (72 x 72 DPI)

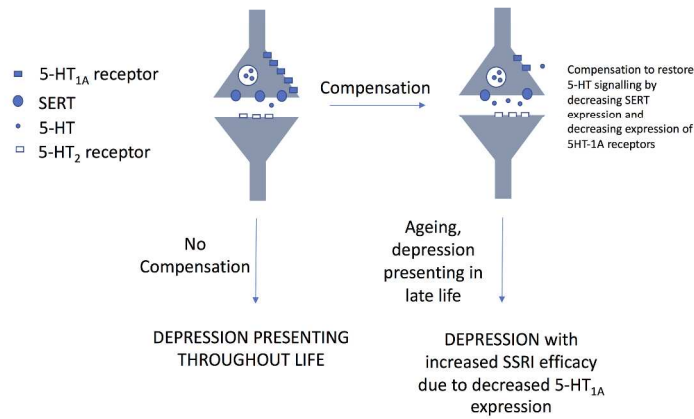


Fig. 5 Proposed mechanism to explain unexpected positive response of GG individuals to citalopram. The GG genotype has been postulated to comprise two sets of individuals, those who possess a compensatory mechanism to restore 5-HT signalling, and those who do not. It might be expected that GG individuals without a compensatory mechanism 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.

338x190mm (300 x 300 DPI)

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-HT_{1A} 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{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 $\Pr(\bar{R}|G,C)$. 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 remission 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 $\Pr(G|\bar{R}, C)$ is the likelihood function for the current genotypic data and $\Pr(\bar{R}|C)$ 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:

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

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

(3)

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18 The values of a and b that define this distribution can be drawn from previously observed
19 data relating to the unconditional probability that an older individual will go into remission
20 following a course of citalopram. For example, the combined remission rates in 6 studies
21 investigating the use of citalopram in late-life depression is calculated at around 47%
22 ($n=567$) (3). In this scenario, $a \approx b$. For example, where $a = b = 4$, the distribution is broadly
23 described as symmetrically bell-shaped around a mean of 0.5 (corresponding to a probability
24 of remission of 0.5), but where probabilities of 0.2 and 0.8 are not unreasonable. If no useful
25 data can be obtained, we take the position that all possibilities for $\Pr(\bar{R})$ are equally likely,
26 which is a specific case of the beta distribution ($a = b = 1$) and defines a non-informative
27 uniform distribution.
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38 To obtain the posterior distribution of relative risk ($\Pr(\bar{R}|CG, C) / \Pr(R|CG, C)$, eq (1)) we
39 applied Gibbs sampling in order to sample from the two distributions ($\Pr(\bar{R}|CG, C)$ and
40 $\Pr(R|CG, C)$), without having to explicitly calculate the integrals. This was performed in R (R
41 3.2.2, 2015) using the script employed in “OPTIMISE trial in a Bayesian framework” (4),
42 which incorporates the scripts “openGraphSaveGraph.R” and “plotPost.R” (2).
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