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SSRI response in depression may be influenced by SNPs in *HTR1B* and *HTR1A*

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Abstract

Desensitization of serotonin 1A (*HTR1A*) and 1B (*HTR1B*) autoreceptors has been proposed to be involved in the delayed onset of response to SSRIs. Variations in gene expression in these genes may thus affect SSRI response. Here we test this hypothesis in two samples from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D), and show evidence for involvement of several genetic variants alone and in interaction. Initially, three functional SNPs in the *HTR1B* gene and in the *HTR1A* gene were analyzed in 153 depressed patients treated with citalopram. QIDS-C scores were evaluated over time with respect to genetic variation. Subjects homozygous for the - 1019 G allele (rs6295) in *HTR1A* showed higher baseline QIDS scores ($p = 0.033$), and by 12 weeks had a significantly lower response rate ($p = 0.005$). *HTR1B* haplotypes were estimated according to previously reported *in-vitro* expression levels. Individuals who were homozygous for the high-expression haplotype showed significantly slower response to citalopram ($p = 0.034$).

We then analyzed more SNPs in the extended overall STAR*D sample. Although we could not directly test the same functional SNPs, we found that homozygotes for the G allele at rs1364043 in *HTR1A* ($p = 0.045$) and the C allele of rs6298 in *HTR1B* showed better response to citalopram over time ($p = 0.022$). Test for interaction between rs6298 in *HTR1B* and rs1364043 in *HTR1A* was significant (overall $p = 0.032$).

Our data suggest that an enhanced capacity of *HTR1B* or *HTR1A* transcriptional activity may impair desensitization of the autoreceptors during SSRI treatment.

Keywords

SSRI; depression; haplotype; *HTR1B*; *HTR1A*; association

INTRODUCTION

Drug response to the first antidepressant administered to a subject with depression is only approximately 50% (Fava *et al* 2003). This high level of non-response results in increased

medical and mental health care increasing costs and personal suffering (Sackeim 2001). The individual variability in response could be due to pharmacokinetic or pharmacodynamic differences that reflect underlying genetic differences (Kirchheiner *et al* 2004). However, neither the neurobiology of mood disorders nor the precise mechanisms of action of the drugs used to treat mood disorders have been completely unraveled. Selective serotonin reuptake inhibitors (SSRIs) are thought to exert their effect by modulating serotonergic activity by inhibiting the serotonin transporter capacity to reuptake serotonin. Although 5-HTT is the main target of SSRIs, the effect of SSRIs on the transporter is immediate, but the delay in onset of its therapeutic effect suggests that other proteins may be involved. A progressive desensitization of serotonin 1A (HTR1A) and 1B (HTR1B) autoreceptors accompanies the delay in the onset of action of SSRIs (El Mansari *et al* 2005; Lifschytz *et al* 2004). Studies in rodents also support desensitization of HTR1A and HTR1B autoreceptors as a key adaptive change in antidepressant action. Internalization and loss of HTR1A autoreceptors has been observed after 2 to 3 weeks of SSRI treatment (Hervas *et al* 2001; Riad *et al* 2001), whereas postsynaptic receptors remain intact. Acute administration of a novel HTR1A/1B autoreceptor antagonist/5-HT transporter inhibitor resulted in two-three fold increase in extracellular 5-HT in cortex of rats and guinea pigs (Hughes *et al* 2007). Furthermore, desensitization of these autoreceptors was observed in rodents upon chronic treatment with transcranial magnetic stimulation (Gur *et al* 2000), electroconvulsive therapy (Gur *et al* 2002a) and other types of antidepressants (Gur *et al* 2002b), suggesting that this is an important mechanism of antidepressant action. Thus, functional genetic variants in *HTR1A* and *HTR1B* may be involved in individual differences in SSRI treatment response.

The functional C-1019G SNP (rs6295) of the *HTR1A* gene (Wu and Comings 1999) is part of a 26 bp imperfect palindrome that binds transcription factors of the repressors/enhancer-type transcriptional regulator (NUDR/DEAF-1). The G allele increases *HTR1A* transcription in some cell types (Lemondé *et al* 2003), and association has been reported with depression- and anxiety disorders (Lemondé *et al* 2003; Rothe *et al* 2004; Strobel *et al* 2003) and with poorer response to antidepressants (Hong *et al* 2006; Lemondé *et al* 2004; Yu *et al* 2006). The CC genotype carriers benefited more from Transcranial magnetic stimulation (TMS) treatment than C/G and G/G in depressed subjects (Zanardi *et al* 2007) and to fluvoxamine in Bipolar patients (Serretti *et al* 2004).

Genetic variation in *HTR1B* has also been extensively analyzed in psychiatric and behavioral traits. A synonymous G allele at position 861 was found to be associated with suicide attempts in personality disorders, bulimia nervosa and obsessive compulsive disorder (Levitan *et al* 2005; Levitan *et al* 2006; Levitan *et al* 2001; Mundo *et al* 2000). The C allele has been found associated with Alcoholism (Lappalainen *et al*, 1998). Recently, additional SNPs have been identified and haplotypes have been tested for functionality: Two SNPs on the promoter region affect transcription levels in reporter gene assays. The -261G_A-161 haplotype showed a 2.3-fold enhanced transcriptional activity compared to the other major haplotypes, indicating that both SNPs are functional and act synergistically. Both sites were shown to modify the binding of transcription factors (Duan *et al* 2003).

Initially, in this study, we investigated the influence of functional polymorphisms in *HTR1A* (G-1019C) and *HTR1B* (T-261G, A-161T and G861C) and seven polymorphisms in seven candidate genes previously investigated in depression and/or SSRI response in 153 depressed patients treated with citalopram. To further investigate the *HTR1A* and *HTR1B* genetic variation influence on citalopram response, we obtained QIDS-C scores and genotyping data for six additional SNPs (other than the SNPs genotyped in the discovery phase) from all 1502 Caucasian patients who participated in the STAR*D study. Our analyses evaluate changes in QIDS-C scores over time as a continuous trait rather than assigning a discrete response status to each subject.

MATERIALS AND METHODS

Subjects

Our exploratory sample consisted of depressed patients selected from the University of Michigan Health System as part of the STAR*D study, a large collaborative project across the US, aiming to compare various treatment options for depression. 210 outpatients diagnosed with non-psychotic major depression participated in a clinical trial of citalopram from which 158 provided a blood sample for this study. Of these, 153 depressed patients completed the trial and were included in this study (age 19–60; females/males 101/52). The majority (125) of subjects were of Caucasian origin (82%), and 28 (18%) belonged to other ethnicities (5 Hispanics, 2 Native Americans, 6 Asian Americans, 15 African Americans).

This study was approved by the University of Michigan Internal Review Board (IRB) and all subjects gave informed consent. Genomic DNA was extracted from blood and lymphocytes were isolated and immortalized by performing Epstein Barr Virus (EBV) transformation for continued DNA supply. All patients were evaluated near baseline and weeks 2, 4, 6, 9, 12 and 14 using the 16-Item Quick Inventory of Depressive Symptomatology Clinician rating (QIDS-C) (Rush *et al* 2003) and treated initially with the SSRI, citalopram. Patients with unsatisfactory response were randomized into different secondary treatment options that are not considered here. All participants initially received citalopram 20–60 mg/day for 12 weeks. Those with clear intolerance or no significant reduction in baseline symptom severity (<15% by week 6 or <25% by week 9) moved to the next treatment level.

Genotype and clinical data for the extended sample consists of 1915 (1502 Caucasian) depressed patients from the STAR*D large sample set, which included most of our exploratory subset, was generated at NIMH by Francis J. McMahon. We received genotype data for six SNPs at *HTR1A* (5q12.2) and *HTR1B* loci (6q13), QIDS-C scores at baseline and weeks 2, 4, 6, 9, 12 and 14 and ethnicity. Detailed demographic and clinical data for this large sample are reported elsewhere (Kraft *et al* 2007; McMahon *et al* 2006). Based on the QIDS-C scores over time in this extended sample, we were able to identify 114 Michigan subjects from the exploratory sample.

DNA analysis

Genomic DNA was extracted from whole blood using the Puregene genomic DNA purification kit (Gentra Systems, Minneapolis, MN, USA). DNA was quantified using the PicoGreen ds quantitation assay (Molecular probes, Eugene, OR, USA) using a Wallac 1420 Victor² fluorescence microplate reader (Perkin Elmer, Boston, MA).

Genotyping

Single Nucleotide Polymorphisms (SNPs)—In the exploratory sample, single nucleotide polymorphisms (SNPs) were genotyped by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis or Taqman (Applied Biosystems), and length repeat polymorphisms by PCR followed by gel-size fragment separation. PCR of 20 microliters (μl) was performed containing 20 ng genomic DNA, 1 U AmpliTaq Polymerase (Applied Biosystems, Foster City, CA), 125 μM each dNTPs (Invitrogen, Carlsbad, CA), 2.5mM MgCl₂, 15mM Ammonium sulphate, 60mM Tris-HCl (pH 8.5) and 500nM each primer, forward and reverse. Primer sequences for most of the variants were reported previously except for *HTR1A* and *TPH2* for which we designed primers using Primer3 software. Samples were cycled using similar protocols for all variants with different annealing temperatures, as follows: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55–61°C for 30 s and 72°C for 30 s with a final 5 min at 72°C. PCR amplification product was detected by agarose-gel electrophoresis. Next, 5 – 6 μl of PCR product was digested for

4 hours with the appropriate restriction enzyme and resolved on agarose gel. Restriction digestions were performed as described previously: for G861C (rs6296) in *HTR1B* (Lappalainen *et al* 1995a), T102C (rs6313) in *HTR2A* (Erdmann *et al* 1996), T1080C (rs6300) in *HTR1D* (Ozaki *et al* 1995), C69G or Ser23Cys (rs6318) in *HTR2C* (Lappalainen *et al* 1995b), and the Val66Met (rs6265) in *BDNF* (Sen *et al* 2003). For C-1019G SNP (rs6295) in *HTR1A* primers were designed (HT1Af-CTGAGGGAGTAAGGCTGGAC; HTR1Ar-GAAGAAGACCGAGTGTGTCTAC) to amplify a 174 bp fragment. Primer HTR1Ar was modified at the second base from the 3' end to allow a restriction site for the enzyme HpyCH4 IV. When the 'C' allele is present two fragments are generated, 153 bp and 21 bp while the 'G' allele remains uncut. Primers for *TPH2* SNP in intron 8 G>A (rs1386483) were designed (TPH2f-GCT GGC TCT GAA CGT GTA TTT TG; TPH2r- TTT GGC TGA TTT TCC TAA TTA AT) resulting in a 142 bp PCR product. The TPH2r PCR primer was modified at the third base from the 3' end to allow a restriction site for the enzyme *SspI* when allele A is present generating two fragments of 122 bp and 20 bp whereas the G allele remained undigested.

Taqman® SNP genotyping assay (Applied Biosystems, Foster City, CA) was used to genotype two promoter SNPs, T-261G (rs11568817) and A-161T (rs130058) in the *HTR1B* gene, one SNP C3029T in intron 1 (rs1360780) of the *FKBP5* gene.

Six SNPs were genotyped at NIMH: A1179G (rs6297), Ser43Ser (rs6298), A-3749G (rs1213366) at the *HTR1B* locus and three SNPs flanking the *HTR1A* gene, from which two (rs1364043 and rs1423691) are located downstream and one (rs10042486) is in the upstream region. SNPs were genotyped either by Taqman, or by the Illumina GoldenGate or Infinium I assay, as described elsewhere (McMahon *et al* 2006). Additional non-synonymous SNPs available in dbSNP for these regions have a heterozygosity of < 0.10 for the Caucasian population and not considered for genotyping.

Length Repeat polymorphism genotyping—5-HTTLPR was genotyped by PCR amplification as described previously (Sen *et al* 2004) with a slight modification. We replaced the Opti-Prime Buffer #6 for the SCB buffer (Baskaran *et al* 1996) to improve the amplification of the "L" allele in heterozygous samples.

Statistical Analysis—Statistical analyses were performed using SPSS for windows (version 12; SPSS, Chicago, IL). Seven QIDS-C scores measurements (baseline, weeks 2, 4, 6, 9, 12 and 14) were observed for each individual.

A random coefficient model was fitted to the data using the linear mixed models (LMM) procedure in SPSS. Genotypes were the independent variables. The dependent variable in the model was QIDS-C score (measured over time from baseline up to week 12, since data are missing for many individuals at week 14), and the intercept and effects of time in the model were allowed to randomly vary from individual to individual. The quadratic model fits our data and it does not have *a priori* assumption of equal weekly variances. The model allows testing for interaction between two variants over time. The model also considered gender, age, number of episodes, ethnicity, and baseline QIDS score as covariates, in order to investigate the fixed effects of these covariates on the QIDS response. All reported *p* values are 2-tailed, and statistical significance was set at *p* < 0.05.

Hardy-Weinberg equilibrium for all genotype frequencies were calculated using χ^2 tests (1 df). Deviation from Hardy-Weinberg equilibrium was found for the SNP in *HTR1A* rs10042486 (*p* = 0.02) in the extended sample.

For SNPs in *HTR1B* and *HTR1A*, pairwise Linkage disequilibrium (LD) was assessed using the Graphical Overview of Linkage Disequilibrium (GOLD) program (Abecasis and Cookson

2000) and haplotypes were estimated using the Partition-Ligation-Expectation-Maximization (PL-EM) algorithm (Qin *et al* 2002).

RESULTS

The time course of citalopram response was investigated using linear mixed models. As expected, a significant effect of time was observed, indicating an overall clinical improvement during the trial. Gender, age, number of episodes and ethnicity did not significantly influence antidepressant response nor the effect of the genetic variation on response. The effects of time and time-squared randomly varied from one subject to another, suggesting individual differences in the effects of time on the response. The addition of time squared improved overall fit of the mixed model. Our results in the exploratory sample do not support the previously reported antidepressant response associations with 5-HTTLPR (Arias *et al* 2003; Kim *et al* 2000; Rausch *et al* 2002; Smeraldi *et al* 1998; Yoshida *et al* 2002; Yu *et al* 2002; Zanardi *et al* 2000), *HTR2A*_T102C (Minov *et al* 2001; Peters *et al* 2004) *TPH2* and *FKBP5* (Binder *et al* 2004).

Genetic variation at the *HTR1A* locus and response to citalopram

The G-1019C SNP at *HTR1A* genotyped in the exploratory sample showed association with response to citalopram over time ($p = 0.024$) (Table 1). The *HTR1A**G/G variant was associated with higher baseline QIDS scores. QIDS scores of these subjects decreased over time to the same degree as the scores of other subjects, but remained higher due to the difference in QIDS baseline score. In addition, at week 12 we observed an even larger difference between subjects homozygotes for the G allele ($F = 5.617$, $p = 0.005$) and the other genotypes. When QIDS baseline was considered as a covariate, the difference in QIDS score at week 12 remained significant ($F = 3.482$, $p = 0.035$), suggesting that both baseline severity and further response are affected by this variant (Figure 2a).

In the extended sample, three SNPs flanking the *HTR1A* gene were genotyped (rs1364043, rs1423691 and rs10042486). Figure 1a shows the relative position with respect to the *HTR1A* gene and the extent of LD for these three SNPs and the SNP rs6295 from the exploratory sample. We found nominal association for rs1364043 with response over time ($p = 0.045$) (Table 1). Homozygotes for the G allele show better response over time than homozygotes for the T allele (Figure 2b). No association was found with SNPs rs1423691 or rs10042486 (Table 1). Three common haplotypes accounted for 98% of the observed haplotypes, resulting in six possible haplotype combinations. Haplotype analysis revealed an association with response over time to citalopram ($p = 0.026$). Specifically, individuals who are homozygous for haplotype B that contains the G allele at SNP rs1364043, showed a better response to citalopram over time than any other haplotype combination (Table 1).

Genetic variation at the *HTR1B* locus and response to citalopram

Subjects with the *HTR1B**G/G variant at G861C showed a slower response to treatment and heterozygotes G/C a better response to treatment over time in the exploratory sample ($p = 0.010$) (Table 2). In light of this result, we genotyped in this sample two functional common SNPs, T-261G and A-161T, located in the promoter region of *HTR1B* and in partial LD with the G861C SNP. These two promoter polymorphisms were previously shown to affect the level of gene expression *in-vitro*: transcription is increased 2.3-fold for the GA haplotype compared to all other common haplotypes (Duan *et al* 2003). We hypothesized that this transcription rate at the *HTR1B* gene may affect SSRI-response since SSRIs were reported to down-regulate and/or desensitize *HTR1B* receptors, facilitating their effects on serotonin neurotransmission (Sari 2004). In the exploratory sample the two promoter SNPs, T-261G and A-161T are in partial LD with each other ($r^2 = 0.467$), and with the G861C SNP ($r^2 = 0.267$ for -261; $r^2 = 0.123$ for

-161). Individual SNP analysis of the time course of drug response showed that homozygosity for the T allele of A-161T was nominally associated ($p = 0.045$) with higher QIDS mean score (Table 2) whereas the *HTR1B*_T-261G was not associated by itself. Therefore, haplotypes for the three SNPs in *HTR1B* were constructed using PLEM (Qin *et al* 2002). Five haplotypes were inferred from which four accounted for 99.3% of all haplotypes (Table 3). Because there is previous *in-vitro* evidence of the effect of these haplotypes on the transcriptional activity of this gene, we classified haplotypes in our sample according to the level of expression previously described (Duan *et al* 2003). Haplotype 4, GAG, corresponded to the 2.3-fold higher expression level. Therefore, we assigned low expression when the subject was homozygous for any haplotype other than 4, medium expression when one haplotype was 4 and high expression to homozygotes for haplotype 4. The LMM test showed association of these “expression associated haplotype combinations” with response to citalopram over time (Figure 3a). Individuals with the high expression haplotype showed a significantly ($p=0.034$) slower response to the SSRI (Table 2). This is consistent with down-regulation of *HTR1B* being required for the drug efficacy, and that this down-regulation is less efficient in high expressing individuals.

In the extended sample, we obtained genotype information for three different *HTR1B* SNPs (rs6297, rs6298 and rs1221366), which are not easily correlated to the expression haplotype. Figure 1b shows the relative position of the SNPs in the *HTR1B* gene and the extent of LD using the r^2 metric. Heterozygotes for rs6298 showed a better therapeutic response over time than TT homozygotes ($p = 0.022$) (Table 2 and Figure 3b).

Genetic interaction of HTR1B and HTR1A SNPs and haplotypes in response to citalopram

While we find the high expression/low repression genotypes of *HTR1A* and *HTR1B* associated with slower response in the exploratory sample, none of the subjects had the combination of G/G for the *HTR1A* and the high expression haplotype for the *HTR1B*. Therefore, interaction cannot be directly estimated in this sample. We observed, however, that individuals carrying both variants that do not promote enhanced transcription of *HTR1A* (C/C or C/G) and *HTR1B* (low or medium haplotypes) genes have lower QIDS scores over time (mean score $\pm$ s.e.m.) (9.579 ± 0.314) than those having either gene polymorphism/haplotype alone (11.641 ± 1.455 for the combined high haplotype in *HTR1B* and C/C or G/C in *HTR1A*; 11.193 ± 0.500 for the combined G/G in *HTR1A* and low or medium in *HTR1B*) (Figure 4a).

When testing interaction in the extended sample between the SNP rs6298 in *HTR1B* and rs1364043 in *HTR1A*, the overall p value is significant ($p = 0.032$); and between TT (rs6298) and TT (rs1364043) the p value is 0.004 with higher QIDS-C mean scores (11.8 ± 0.4) than either one alone (10.32 ± 0.372 for rs6298; 10.912 ± 0.120 for rs1364043) (Figure 4b). The lowest QIDS-C mean scores was observed for rs6298 CC and rs1364043 GG (9.7 ± 0.6).

DISCUSSION

We investigated the contribution of several polymorphisms in serotonin-related candidate genes to the response of depressed patients over time treated with citalopram. This approach differs from other studies where the response criterion is dichotomized based on depression scores after few weeks of treatment. In this case, we evaluate depression scores quantitatively along the treatment period of 12 weeks. Our results in the exploratory sample do not support the previously reported antidepressant response associations with 5-HTTLPR (Arias *et al* 2003; Kim *et al* 2000; Rausch *et al* 2002; Smeraldi *et al* 1998; Yoshida *et al* 2002; Yu *et al* 2002; Zanardi *et al* 2000), *HTR2A*_T102C (Minov *et al* 2001; Peters *et al* 2004) *TPH2* and *FKBP5* (Binder *et al* 2004). Most likely our small sample size may not have the power to detect an effect. In addition, there were differences in study design - not all of the previous studies used citalopram, and response criteria differ significantly among studies. Nevertheless, for the

most frequently discussed and published variant, 5-HTTLPR, we find not even a trend of an effect on citalopram response.

Instead, we find association with both *HTR1A* and *HTR1B* genes that encode two receptors which are downregulated after SSRI treatment (Sari 2004). Our results although nominal and not corrected for multiple testing suggest that higher baseline level of expression of these two receptors interferes with SSRI efficacy: First, the *HTR1A* variant associated with lower response, i.e. the G allele of C-1019G, prevents binding of a repressor (NUDR/DEAF-1) in the raphe (Lemondé *et al* 2003). The same allele has been found associated with poorer response to antidepressants in two other studies (Hong *et al* 2006; Lemondé *et al* 2004). Furthermore, the G allele was associated with depression (Lemondé *et al* 2003), anxiety- and depression-related personality traits (Strobel *et al* 2003), agoraphobic subtype of panic disorder (Rothe *et al* 2004). Our results are consistent with these findings, since we find association both with higher QIDS scores (i.e. depression severity) throughout the trial, but also independently with response at week 12 ($p=0.035$) while accounting for QIDS scores at baseline as a covariate. Combining the two effects, i.e. not taking initial QIDS score into account, as is done in most previous association studies on response, yields highly significant response difference ($p = 0.005$), which remains significant after accounting for multiple testing.

Second, after finding a nominal association between homozygosity for the G allele on *HTR1B*_G861C and non-response, we tested two functional SNPs in the promoter in the exploratory sample, and found that a haplotype combination of SNPs that has previously been reported to increase transcription (Duan *et al* 2003) was significantly associated with slower response to the SSRI citalopram. However, only four subjects were homozygous for haplotype 4, three of which maintained high scores over time, and one reached a QIDS score of 5, i.e. barely passed remission criterion, at week 12. Our data suggest that the higher transcriptional level may interfere with the downregulation previously found necessary for SSRI efficacy (Sari 2004). Both genes are integral to the negative feedback control of serotonergic activity (Lifschytz *et al* 2004). Interaction between these two genes could not be estimated in the exploratory sample because the combination of both the G/G in *HTR1A* and the high expressed haplotype in *HTR1B* was not present in this sample. However, individuals with at least one of the genotypes that increased transcription (in either *HTR1A* or *HTR1B*) had higher QIDS scores over time than individuals with common variants for both genes (who showed the best response to citalopram; 106/147; 72%; see Figure 4a). If these genetic variants were at least additive, one might speculate that individuals with high levels of transcription in both genes might be particularly treatment resistant, a hypothesis that can be tested in larger samples. Our findings, while clearly preliminary, would predict that genetic variants enhancing transcription may affect desensitization of both receptors by the SSRI and ultimately drug response. This hypothesis is supported by pharmacological data showing that an antagonist for both autoreceptors accelerates the onset of therapeutic effect (Starr *et al* 2007). Testing of this hypothesis in a large sample is warranted.

However, there are two caveats to our results. First, only four out of 153 subjects were homozygotes for the highly expressed haplotype – and these all were not or barely responsive to citalopram. Second, haplotype phase cannot be inferred unambiguously for double heterozygote subjects for the two promoter SNPs. The PL-EM algorithm defines phase based on the maximum expectation, and double heterozygous samples thus fell into the low expression category. However, given allele frequency, about 5–6% of these double heterozygotes would be expected to have one of the rarer haplotype on one chromosome together with the high expressing haplotype on the other chromosome, and are thus misclassified as low expressing. However, the association with high expression category remains unaffected by this phase uncertainty.

To further explore the genetic variation influence of these two genes, we obtained genotype data for three SNPs from each gene, *HTR1A* and *HTR1B*, from the extended sample of patients participating in the STAR*D study, which includes most of our exploratory sample (McMahon *et al* 2006). As described by McMahon (McMahon *et al* 2006) SNPs were selected from HapMap and none of our functional SNPs were genotyped. For *HTR1A*, the three SNPs genotyped in the large sample flank the gene at about 5 kb on each side. Individuals homozygous for the G allele at rs1364043 and homozygous for a haplotype containing this allele showed better response over time to citalopram. This SNP is located 6.7 kb downstream from *HTR1A* gene and no information is currently available about its functionality or affect on expression. Given that LD between rs1364043 and rs6295 (the C-1019G found associated in the exploratory study) is weak ($r^2 = 0.267$), we cannot conclude that this association is due to this latter, functional SNP. But both data sets independently support that genetic variation at the *HTR1A* locus may be involved in the response to SSRIs.

Similarly, for *HTR1B* we found association for SNP rs6298 in the extended sample. According to HapMap, this SNP is in complete LD ($r^2=1$) with the coding SNP rs6296 found associated in our exploratory sample. Heterozygotes for both SNPs showed better response to citalopram over time than either type of homozygotes. For rs6298, homozygotes for the T allele show higher QIDS scores over time. Unfortunately, we cannot determine whether or not the observed association in the extended sample is due to the promoter variants, since rs6298 alone cannot tell the high expressed haplotype apart. It appears that in *HTR1B* there are at least two functional variants on several haplotype backgrounds. Clearly, testing more SNPs including the two promoter variants in a larger sample may help elucidate the contribution of these genes to antidepressant response.

A significant interaction between the two associated SNPs from each gene, *HTR1A* and *HTR1B*, was observed in the extended sample. Individuals homozygous for the T allele at both loci have higher mean QIDS scores over time than either one alone, suggesting a synergetic (more than additive) effect on the response to treatment. This combination was even more significantly associated with antidepressant response ($p = 0.004$) than any other SNP combination and the global interaction was also significant ($p = 0.032$).

In this same line of evidence, novel therapeutic approaches include a selective HTR1A/1B autoreceptor antagonist and 5-HT re-uptake inhibitor for a faster antidepressant action (Scott *et al* 2006) and acute anxiolytic effect in rats (Starr *et al* 2007). When adding a HTR1A antagonist to an SSRI, a faster improvement was observed in some studies (Artigas *et al* 1994; Blier and Bergeron 1995) but not reproduced by others (Berman *et al* 1999; Berman *et al* 1997) and a recent meta-analysis confirmed the faster improvement in the first two week period but not beyond that period (Ballesteros and Callado 2004). A substantial increase in 5-HT has been observed when combining an SSRI with a HTR1A/1B antagonist compared to using HTR1A antagonists alone (Gobert *et al* 2000; Sharp *et al* 1997). Recently a study on the transcriptional regulation at *HTR1A* C(-1019)G suggested that genetic heterogeneity may obscure the association of this functional polymorphism on mood a suggestion that is further supported by the several reports that find associations when gene-gene or gene-environment interactions are considered (Le Francois *et al* 2008).

Conclusion

In summary, we investigated the natural course of response to SSRIs over time in relation to genetic variation at two SSRI target genes, an approach that allows the effect of time to randomly vary from individual to individual. Although not conclusive, we show that more than one polymorphism in a gene may contribute to the response to treatment and a more dense analysis in a large sample may help elucidate this influence. Our major finding is the interaction between the two associated SNPs in each gene, *HTR1A* and *HTR1B* where genetic variation at

both loci influences the individual response to treatment, a response tested by the mixed linear model that takes into account inter-individual variability. This is consistent with preclinical studies that show that simultaneous use of antagonists for both genes accelerates and improves the effect of SSRIs.

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a)

b)

Figure 1. Locations of SNPs studied relative to the gene (black box) of a) *HTR1A* and b) *HTR1B* loci. SNPs depicted in the upper side were genotyped in the extended sample and SNPs in the lower part were genotyped in the exploratory sample (not to scale).

Figure 2.

Pattern of QIDS scores during treatment in relation to *HTR1A* genotype. a) In the exploratory sample, homozygous for the G allele of C-1019G *HTR1A* (rs6295) show higher mean scores at baseline ($p = 0.033$), remain high over time ($p = 0.035$) compared to the other two genotypes (G/C and C/C); b) In extended sample, homozygous for the G allele in rs1364043 show better response over time ($p = 0.045$) than G/T and G/G.

Figure 3.
Pattern of QIDS-C scores during treatment in relation to *HTR1B* variants a) haplotypes in exploratory sample and b) rs6298 in extended sample.

a)

	Low/medium HTR1B haplotype	High HTR1B haplotype
Low/medium HTR1A (C/*)	Responder QIDS = 9.6 +/- 0.3 N=106	Less response QIDS = 11.6 +/- 1.5 N=4
High HTR1A (GG)	Less response QIDS = 11.2 +/- 0.5 N=37	Predicted to be even less responsive N/A

b)

		HTR1B rs6298		
		TT	CT	CC
HTR1A rs1364043	TT	<u>11.8 ± 0.4</u> n = 73	11.1 ± 0.2 n = 331	11.1 ± 0.2 n = 483
	GT	11.4 ± 0.5 n = 47	<u>10.3 ± 0.3</u> n = 195	11.1 ± 0.2 n = 271
	GG	11.1 ± 1.8 n = 5	10.4 ± 0.7 n = 32	<u>9.7 ± 0.6</u> n = 41

Figure 4.

HTR1A and *HTR1B* combined data according to level of transcription and interaction. a) In the exploratory sample, subjects with low/medium expression variants for both genes showed lower QIDS scores than subjects with at least one high expression variant; b) In the extended sample, interaction between rs1364043 at *HTR1A* and rs6298 at *HTR1B* was significant ($p = 0.032$). Homozygotes individuals for the T allele at both loci showed higher mean QIDS scores than either one alone.

Table 1
Mixed model of the response to citalopram for *HTR1A* SNPs and haplotypes.

SNP	Genotypes	N (frequency)	EMM ^a	s.e.m.	P value
rs1364043 ^b	TT	902 (0.60)	10.912	0.120	0.045
	GT	522 (0.35)	10.559	0.155	
	GG	78 (0.05)	10.113	0.395	
rs1423691 ^b	TT	409 (0.27)	10.975	0.175	0.118
	CT	724 (0.48)	10.853	0.134	
	CC	369 (0.25)	10.484	0.184	
rs10042486 ^b	TT	370 (0.25)	10.550	0.184	0.222
	CT	691 (0.47)	10.816	0.136	
	CC	406 (0.28)	10.976	0.175	
Haplotypes	TCT	2N = 3004			
	GCT	784 (0.26)			
	TTC	676 (0.22)			
	others	1,532 (0.50)			
Combined haplotype	no B haplotype	12 (0.02)			
	1 B haplotype	906 (0.60)	11.005	0.117	0.026
	2 B haplotypes	521 (0.35)	10.661	0.147	
rs6295 ^c	CC	75 (0.05)	10.194	0.368	0.024
	GC	45 (0.29)	8.318	0.552	
	GG	69 (0.45)	8.207	0.447	
		39 (0.26)	10.133	0.594	

^a Estimated marginal means over time
^b SN Ps genotyped in extended sample
^c SN P genotyped in exploratory sample
^d haplotype bearing the G allele at rs1364043

Table 2

Mixed model of the response to citalopram for *HTR1B* SNPs and haplotypes.

SNP	Genotypes	N (frequency)	EMM ^a	s.e.m.	P-value
rs11568817 ^b	TT	49 (0.32)	8.51	0.463	0.459
	TG	70 (0.46)	8.41	0.393	
	GG	32 (0.21)	9.24	0.566	
rs130058 ^b	AA	77 (0.52)	8.91	0.368	0.045
	AT	59 (0.40)	8.09	0.422	
	TT	11 (0.08)	10.52	0.938	
rs6296 ^b	GG	82 (0.54)	9.39	0.352	0.01
	GC	60 (0.39)	7.78	0.397	
	CC	11 (0.07)	8.46	0.904	
rs6297 ^c	AA	1296 (0.871)	10.83	0.103	0.679
	AG	183 (0.123)	10.59	0.259	
	GG	9 (0.006)	10.94	1.151	
rs6298 ^c	CC	795 (0.54)	9.58	0.148	0.022
	CT	558 (0.38)	9.23	0.177	
	TT	125 (0.08)	10.32	0.372	
rs1213366 ^c	AA	463 (0.31)	11.00	0.165	0.191
	AG	726 (0.48)	10.64	0.133	
	GG	312 (0.21)	10.88	0.200	
Haplotypes ^b Low Medium High	No haplotype 4	104 (68.9)	8.59	0.319	0.034
	1 haplotype 4	43 (28.5)	8.29	0.485	
	2 haplotype 4	4 (2.6)	12.48	1.520	

^aEstimated QIDS-C marginal means over time
^bSNPs genotyped in exploratory sample. Haplotype 4 from table 4
^cSNPs genotyped in extended sample

Table 3

Frequencies of *HTR1B* haplotypes.

SNPs	rs1213366 A-3749G	rs11568817 T-261G	rs130058 A-161T	rs6298 T129C	rs6296 G861C	Frequency
Haplotypes in HapMap						
	A	T	A	T	C	0.342
	A	T	A	C	G	0.242
	G	G	T	C	G	0.208
	G	G	A	C	G	0.175
	A	G	A	C	G	0.017
Haplotypes in exploratory sample						
1		T	A		C	(2N = 302)
2		G	T			0.275
3		T	A		G	0.268
4		G	A		G	0.281
5		G	T		G	0.169
Haplotypes in exploratory sample						
6	G				C	0.007
7	A			C		(2N = 3,000)
8	A			C		0.448
				T		0.278
						0.272

Transcription *in-vitro*^b

1
1.034
1
2.319
1.034

^b (Duan *et al* 2003)