The C(–1019)G polymorphism of the 5-HT1A gene promoter and antidepressant response in mood disorders: preliminary findings

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Abstract

Several studies have demonstrated the involvement of 5-HT1A receptors in the pathogenesis of depression and in the antidepressant response to SSRIs. A functional new variant in the promoter region of the 5-HT1A gene was recently reported (–1019 C>G). The aim of this study is to investigate a possible association between this 5-HT1A receptor variant and antidepressant response to fluvoxamine in a sample of 262 mood-disorder subjects (151 major depressed and 111 bipolars) treated with fluvoxamine for 6 wk. The severity of depressive symptoms was assessed weekly with the Hamilton Rating Scale for Depression (HAMD). 5-HT1A variants did not influence antidepressant response in the whole sample and in unipolar subjects. In bipolars, 5-HT1A*C/C genotype carriers showed a better response to fluvoxamine (p = 0.036), independently from clinical variables. The 5-HT1A polymorphism effect on antidepressant response was independent from the previously reported effect of the 5-HTTLPR polymorphism. In conclusion, 5-HT1A variants could influence the antidepressant efficacy in bipolar subjects, even if results must be verified on larger samples.

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Key words: Antidepressant treatment, bipolar disorder, genetics, major depressive disorder, pharmacogenetics.

Introduction

The functional polymorphism in the upstream regulatory region of the serotonin transporter (5-HTTLPR) gene was repeatedly associated with antidepressant response to selective serotonin reuptake inhibitors (SSRIs) in mood disorders (Arias et al., 2001; Pollock et al., 2000; Smeraldi et al., 1998; Yu et al., 2002; Zanardi et al., 2000, 2001b). Preliminary results also suggest that the tryptophan hydroxylase (TPH) gene variants (A218C) were involved in the outcome of antidepressant treatment (Peters et al., 2003; Serretti et al., 2001a,b), even if those findings were not univocally confirmed (Kim et al., 2000; Yoshida et al., 2002). Further not univocally replicated positive findings included G-protein beta3-subunit (Gb3) and serotonin receptor 2A gene polymorphisms (Cusin et al., 2002; Minov et al., 2001; Serretti et al., 2003; Zill et al., 2000).

In the search for further genes influencing response, we focused on the 5-HT1A receptor. Electrophysiological and microdialysis studies performed on animals have shown that administration of SSRIs causes a functional blockade of serotonin (5-HT) feedback onto somatodendritic 5-HT1A receptors. 5-HT neurons therefore continue firing and synthesizing 5-HT, while SSRIs increase synaptic 5-HT concentration through the uptake-pump block (SERT), thus facilitating 5-HT neurotransmission (Bel and Artigas, 1993; Sprouse et al., 2001); this interaction could represent the origin of antidepressant response. A number of partial agonists of the 5-HT1A receptor have been shown to exert a synergic action with 5-HT reuptake blockers in the treatment of depression (Albert et al., 1996; Artigas et al., 1996; Blier and de Montigny, 1994; Mongeau et al., 1997; Zanardi et al., 1997, 1998). Pindolol, a beta adrenoceptor antagonist that also has 5-HT1A receptor antagonist properties, has been used to accelerate the onset of antidepressant action...
by blocking 5-HT1A pre-synaptic receptors (Perez et al., 1997).

Serotonin 1A (5-HT1A) receptors are located both at a post-synaptic and at a pre-synaptic level; in the first case, they mediate the action of 5-HT on cortical and limbic neurons and are thought to play an important role in the pathogenesis of depressive symptomatology, in the second case, they act as serotonergic autoreceptors on serotonergic neurons in the raphe nuclei and prevent the release of 5-HT by a negative feedback (Dubovsky and Thomas, 1995; Kapur and Remington, 1996). 5-HT1A pre-synaptic receptors exert a self-inhibitory function and when they are stimulated by 5-HT the result is a decrease in neuronal firing, and 5-HT synthesis and release.

The 5-HT1A receptor gene was mapped on the long arm of chromosome five (5q11.2-13) and it appears to be intronless (Kobilka et al., 1987). It contains an uninterrupted long open reading frame encoding a G protein-coupled receptor, that acts primarily via inhibition of adenylate cyclase. A functional new variant in the promoter region of the gene was recently reported (Wu and Comings, 1999). This polymorphism consists of a G to C substitution and is located at position 92 928 bp (GDB: AC008965) of the human 5-HT1A gene. It is inside a palindromic region of 26 bp, which bounds a single repressor, the so-called Nuclear DEAF-1-related (NUDR) protein (Lemonde et al., 2003). This variant was demonstrated to be involved in modulating the rate of transcription of the 5-HT1A gene. When the G-allele is incorporated, it prevents the binding of this putative repressor to DNA, leading, in this way, to an increase of 5-HT1A autoreceptors and a reduction of serotonergic neurotransmission (Stahl, 1994). This C→G polymorphism of the 5-HT1A promoter was associated with a number of psychiatric disorders including major depression, suicide and anxiety-related traits (Lemonde et al., 2003; Rothe et al., 2004; Strobel et al., 2003).

To our knowledge, the association between this polymorphism and antidepressant response has not been investigated to date. The aim of our study is to investigate a possible association between these variants of the 5-HT1A receptor and the antidepressant response to fluvoxamine in a sample of 262 depressed subjects treated with fluvoxamine.

Materials and methods

Sample

A total of 262 in-patients affected by major recurrent depression and bipolar disorder admitted to the Mood Disorder Centre at the Department of Psychiatry of San Raffaele Hospital, Milan were included in the study. Lifetime diagnoses were assigned according to DSM-IV criteria (APA, 1994) on the basis of structured clinical interviews, the Schedule for Affective Disorder and Schizophrenia (SADS; Endicott and Spitzer, 1978) and/or the Structured Clinical Interview for DSM-IV (SCID; First et al., 1995), plus all available sources. A first psychiatrist evaluated the retrospective course of illness, by interviewing subjects, family members, previous health professionals and obtaining records where possible (Leckman et al., 1982). A second experienced psychiatrist reviewed the chart and, if no consensus was obtained, a third senior psychiatrist was involved. However, no subject was excluded because of disagreement.

The sample is described in Table 1. Inclusion criteria were described elsewhere (Smeraldi et al., 1998; Zanardi et al., 2000, 2001). The sample was previously analysed for association between antidepressant treatment and other candidate genes in published studies (Cusin et al., 2002; Serretti et al., 2001b; Smeraldi et al., 1998; Zanardi et al., 2001); the amount of drop-out patients in those studies was 12 subjects and they have been described previously; the small drop-out rate is due to the in-patient setting of the studies. The presence of any concomitant Axis I diagnosis and somatic or neurological illnesses impairing psychiatric evaluation represented exclusion criteria. All patients were evaluated at baseline and weekly thereafter until the sixth week using the 21-item Hamilton Rating Scale for Depression (HAMD-21; Hamilton, 1967) administered by trained senior psychiatrists blind to genetic data.

Subjects for the present study have been treated as described in our previous antidepressant trials and under double-blind conditions (Smeraldi et al., 1998; Zanardi et al., 2001). Briefly, after a 7-d washout period, fluvoxamine was titrated to reach 300 mg/d. Concomitant psychotropic drugs were not allowed, except lithium maintenance and flurazepam at bedtime (up to 45 mg). A decrease in HAMD scores to ≤8, with Delusion factor equal to 0 (items 2, 15, 20) (Bech et al., 1993; Bellini et al., 1992; Sobin and Sakheim, 1997), was considered the response criterion. After the procedure had been fully explained to all subjects, informed consent was obtained.

Plasma fluvoxamine levels were determined by high-performance liquid chromatography after 2 wk of stable daily dose (Lucca et al., 1994). Patients who showed fluvoxamine plasma levels exceeding the mean value of the sample ±2-fold the standard deviation were originally not included to avoid the...
possibility that extreme differences in the bioavailability of the drug could influence the clinical response. We excluded from our study individuals who reached a HAMD-21 score decrease of more than 50% of the baseline value after the first week of treatment, to avoid the presence of ‘placebo responder’ individuals (Quitkin et al., 1984, 1987; Rausch et al., 2002), or of spontaneous remissions. Nineteen subjects were excluded from the analysis for this reason. However, they did not differ from the total sample in terms of clinical characteristics and genotype frequencies (data not shown).

**DNA analysis**

Genomic DNA was extracted from leucocytes by NaCl precipitation (Lahiri and Nurnberger, 1991). PCR was performed with the following primers: 5’-CCCAGAGTGGCAATAGGAGA-3’ and 5’-CCGTTTTGTTGTTGTTGCG-3’. The PCR reaction was carried out in a 10 ml volume containing 150 ng genomic DNA, 5 pmol of each primer, 200 mM each dNTP, 1× PCR Gold Buffer (Applied Biosystems, Monza, Italy), and 0.025 U/ml of Taq Gold Polymerase (Applied Biosystems). After an initial step of 5 min at 95 °C, 35 cycles of amplification (30 s at 95 °C, 30 s at 62 °C, 45 s at 72 °C) and a final extension step of 10 min at 72 °C were performed. Then, after purification of PCR product, we performed a SnaPshot ddNTP Primer Extension (kit by Applied Biosystems). The extension reaction was carried out with the 5’-GGAAGAAGACCGAGTTGCTTCG-3’ primer (10 μM) and with the

| Table 1. Genotype frequencies and clinical and demographic variables (the number of subjects for which the information was available is in parentheses) |
|-----------------|-------|-------|-------|-------|-------|-----|-----|
| Variables (5-HT1A) | C/C   | C/G   | G/G   | Total sample | F    | p   |
| Age (yr) (259) | 48.83 ± 12.83 | 52.93 ± 12.56 | 49.83 ± 14.85 | 51.15 ± 13.33 | 2.44 | 0.08 |
| Onset (yr) (257) | 34.72 ± 11.46 | 38 ± 13.40 | 35.86 ± 14.58 | 36.68 ± 13.30 | 1.43 | 0.24 |
| Total no. of episodes (218) | 5.45 ± 5.32 | 4.94 ± 5.29 | 4.71 ± 5.49 | 5.01 ± 5.33 | 0.26 | 0.76 |
| HAMD-21 score at baseline (262) | 29.82 ± 5.78 | 30.42 ± 6.60 | 29.53 ± 5.41 | 30.05 ± 6.12 | 0.53 | 0.58 |
| HAMD-21 score at week 6 (262) | 7.93 ± 10.41 | 9.64 ± 12.02 | 9.35 ± 10.80 | 9.15 ± 11.31 | 0.47 | 0.61 |
| Fluvoxamine plasma level (mequiv./l) (150) | 326.55 ± 176.20 | 320.13 ± 285.78 | 258.61 ± 180.22 | 307.90 ± 239.06 | 0.94 | 0.39 |

<table>
<thead>
<tr>
<th>Variables (5-HT1A)</th>
<th>C/C</th>
<th>C/G</th>
<th>G/G</th>
<th>Total</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>38/24</td>
<td>90/43</td>
<td>45/22</td>
<td>262</td>
<td>0.81</td>
<td>0.66</td>
</tr>
<tr>
<td>Diagnosis (MD/BP)*</td>
<td>32/30</td>
<td>72/61</td>
<td>47/20</td>
<td>262</td>
<td>5.88</td>
<td>0.05</td>
</tr>
<tr>
<td>Responders (yes/no)</td>
<td>38/24</td>
<td>80/53</td>
<td>39/28</td>
<td>262</td>
<td>0.13</td>
<td>0.93</td>
</tr>
<tr>
<td>Delusional features (yes/no)</td>
<td>24/30</td>
<td>58/62</td>
<td>26/34</td>
<td>234</td>
<td>0.48</td>
<td>0.78</td>
</tr>
<tr>
<td>SERPR genotypes (ll/ls/ss)</td>
<td>21/21/19</td>
<td>50/49/30</td>
<td>16/30/18</td>
<td>254</td>
<td>4.79</td>
<td>0.30</td>
</tr>
<tr>
<td>Variables (5-HTTLPR)</td>
<td>l/l</td>
<td>l/s</td>
<td>s/s</td>
<td>F</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine plasma level (mequiv./l) (150)</td>
<td>283.89 ± 210.85</td>
<td>314.06 ± 202.98</td>
<td>325.27 ± 306.45</td>
<td>0.36</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

* MD, Major depression, BP, bipolar.
following steps: 10 s at 96 °C, 5 s at 60 °C and 30 s at 60 °C, for 25 cycles. The product was then genotyped by a Genetic Analyser (ABI PRISM® 310, Applied Biosystems), after a denaturation step (95 °C for 5 min).

5-HTTLPR genotyping was also performed, as described elsewhere (Serretti et al., 2002), producing long (528 bp) and short (484 bp) variants.

**Statistical analysis**

Seven HAMD score measurements (at baseline and for 6 wk) were analysed. Multivariate analysis of variance (MANOVA) for repeated measures was used to examine the differences between genotypes on HAMD scores during the 6 wk of treatment. An ‘intent-to-treat’ analysis was carried out for all patients who had a baseline assessment and at least one assessment after randomization, with the last observation carried forward on the HAMD. Student’s t test, ANOVA and $\chi^2$ were used when appropriate. Analysis of covariance (ANCOVA) was used to investigate possible stratification effects. MANOVA for repeated measures including 5-HT1A and 5-HTTLPR variants as main factors was used to investigate a possible interaction between the two polymorphisms. All $p$ values were two-tailed, and statistical significance was set at the 5% level ($p < 0.05$). With these parameters, for continuous measurements, our sample had a high power (0.80) to detect a small effect size ($d = 0.35$), which corresponded to a difference of approximately 3.7 points on the final HAMD score between two genotypes (Cohen, 1988). Statistical analyses were performed using the STATISTICA package (StatSoft, 2004).

**Results**

Genotype frequencies were respectively: C/C 62 (23.67%), C/G 133 (50.76%), G/G 67 (25.57%). They resulted similarly to the frequencies obtained by Lemonde et al. (2003) for their samples of depressed patients [C/C 30 (23.25%), C/G 63 (48.84%), G/G 36 (27.91%)]. The sample resulted in Hardy–Weinberg equilibrium for the analysed polymorphism. The three genotypes did not significantly differ among one another for diagnosis, sex, age, age of onset, presence of psychotic features lifetime, presence of familiarity for mood disorders, number of episodes and Hamilton scores at baseline. Descriptive variables, subdivided according to diagnosis of the subjects are shown in Table 2.

When analysing the whole sample, the three genotypes did not show any difference in antidepressant response (MANOVA: main effect 5-HT1A, $p = 0.88$; time, $p < 0.0001$; interaction 5-HT1A x time, $F = 0.31$, d.f. = 12, 1548, $p = 0.98$), and the same negative finding was observed when analysing unipolar subjects only (MANOVA: main effect 5-HT1A, $p = 0.25$; time, $p < 0.0001$; interaction 5-HT1A x time, $F = 1.42$, d.f. = 12, 882, $p = 0.036$). Among bipolars, including age and sex as covariants, the effect remained significant ($p = 0.036$ in both cases), while the significance was marginally decreased when basal HAMD was included as a covariant (MANCOVA with HAMD-21 score at baseline; bipolar only: $F = 1.70$.

**Table 2. Clinical and demographical variables according to diagnosis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>MD</th>
<th>BP</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.20 ± 12.66</td>
<td>49.74 ± 14.10</td>
<td>1.47</td>
<td>0.14</td>
</tr>
<tr>
<td>HAMD-21 score at baseline</td>
<td>29.46 ± 5.81</td>
<td>30.86 ± 6.46</td>
<td>-1.83</td>
<td>0.07</td>
</tr>
<tr>
<td>HAMD-21 score at week 6</td>
<td>9.15 ± 10.85</td>
<td>9.14 ± 11.95</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Fluvoxamine plasma level (mequiv./l)</td>
<td>308.03 ± 273.63</td>
<td>307.70 ± 181.14</td>
<td>0.008</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>MD</th>
<th>BP</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>107/44 (70.86/29.14%)</td>
<td>66/45 (59.46/40.54%)</td>
<td>3.70</td>
<td>0.05</td>
</tr>
<tr>
<td>Responders (yes/no)</td>
<td>85/65 (56.95/43.05%)</td>
<td>71/40 (63.96/36.04%)</td>
<td>1.30</td>
<td>0.25</td>
</tr>
</tbody>
</table>

MD, Major depression, BP, bipolar, UP, unipolar.
Interaction with 5-HTTLPR

We then considered, within our sample, a possible interaction with 5-HTTLPR variants. We performed a MANOVA for repeated measures including 5-HT1A and 5-HTTLPR variants as main factors. Given that the inclusion of all the genotypes would incorrectly imply a strict co-dominant effect and that the inclusion of all four of the dummy variables (5-HT1A*C/C, 5-HT1A*C/G, 5-HTTLPR*s/s, 5-HTTLPR*l/s – the remaining genotypes are implicit) were not feasible for the sample size, we repeated the analysis including two terms in turn. For example we report the results for the 5-HT1A*C/C and 5-HTTLPR*s/s variants which evidenced a slightly higher significance for the 5-HT1A variant (main effect, \( p = 0.08 \); interaction with time, \( p = 0.018 \)) but no effect of the interaction term (\( p = 0.37 \) and \( p = 0.93 \) respectively). Similar results were obtained for the other combinations (data not shown).

Discussion

This is, to our knowledge, the first association study that investigates antidepressant response according to C(−1019)G 5-HT1A variants. Our preliminary findings on the sample of bipolar subjects suggest a liability effect of this polymorphism on antidepressant efficacy. In fact, bipolar disorder is thought to have a heavier genetic load than unipolar depression (Tsuang and Faraone, 1990). According to the molecular hypothesis, we found that *G-allele-containing individuals showed a worse response to fluvoxamine. Following the hypothesis of polygenic inheritance of complex traits, we investigated a possible interaction with 5-HTTLPR variants, but we found no significant effect. We can provisionally regard the 5-HT1A variants’ influence as independent from 5-HTTLPR. Obviously, we observed this at a clinical level and we cannot infer possible interactions at the molecular level. We have previously observed that pindolol, a partial 5-HT1A antagonist, influenced the antidepressant response, cancelling the influence of the 5-HTTLPR polymorphism. In fact, with pindolol augmentation, the individuals carrying the *s/s genotype, which was usually associated to a worse response, showed a better outcome and it overwhelmed the differences between genotypes (Smiraldi et al., 1998). In the present paper we excluded subjects treated with pindolol in order to avoid ambiguous interpretation of data (plasma levels of pindolol, rationale of the observed combined effect of pindolol plus antidepressant and so on).

In our sample the 5-HTTLPR frequencies were respectively: l/l 87 (34.25%), l/s 100 (39.37%) and s/s 67 (26.38%). They were similar to the frequencies reported in previously published studies in Caucasian patients which, for the s/s genotype, ranged from 21.6 to 28.3% (Smits et al., 2004). Frequencies were also similar for the other two genotypes.

Other factors associated to mood disorders could hide the effect of genes on antidepressant response, or enhance it. In particular, there is evidence that 5-HT1A receptor density might differ according to gender and age (Cidis Meltzer et al., 2001; Parsey et al., 2002), however, genotype frequencies did not differ according to those two variables in our sample.

The main limitation of this study is the small number of subjects which did not allow detection of small differences between genotypes. A further limitation could be represented by the case-control approach. Genomic control strategies are routinely used in order to detect ethnic stratification biases (Pritchard and Rosenberg, 1999), however, our sample was selected among subjects with northern Italian antecedents for at least two generations, being from north Italy is characterized by a substantial genetic homogeneity (Barbujani and Sokal, 1991). Moreover, our centre is a tertiary care setting and, therefore, we...
cannot exclude a potential bias associated with the severity of illness.

Another issue needs to be considered: the presence of placebo response, which was recently associated to 5-HTTLPR genotype (Walsh et al., 2002). This placebo response could in fact reduce the power to detect the real interaction between gene variants and antidepressant response. However, we tried to avoid this effect with the washout period and the exclusion of early responders (Quitkin et al., 1984).

In conclusion we observed a moderate liability effect of 5-HT1A variants in antidepressant response in bipolar disorder but not in major depressives, this finding adds an important piece of information for the pathway of detecting the genetics of antidepressant response.

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Statement of Interest
None.

References


