Association of serotonin-1A and -2A receptor promoter polymorphisms with depressive symptoms, functional recovery, and pain in patients 6 months after lumbar disc surgery

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Abstract

Single nucleotide polymorphisms (SNPs) in the serotonergic (5HT) system seem to have modulatory effects on depression and physical function. Preliminary evidence suggests that gene × environment interactions play a role in the development of depression, with somatic complaints serving as environmental stressors. We hypothesized that pain intensity may serve as a stress factor that modulates the association between SNPs in the 5HT system and depression. We investigated symptoms of pain, depression, physical functioning, and disability in 224 patients 6 months after lumbar disc surgery. Associations between these variables and functional promoter SNPs in the serotonin receptor genes 5HTR1A (rs6295) and 5HTR2A (rs6311) were analyzed. For 5HTR2A, we found a significant gene × environment × sex interaction, as female patients carrying at least one A allele of the 1438A/G promoter SNP had significantly higher depression scores when confronted with severe pain compared to women harboring the GG genotype (P = .005). For 5HTR1A, patients homozygous for the 1019 G allele presented higher Beck Depression Inventory scores relative to the CG/CC group, indicating a major effect of this SNP on depression. Furthermore, women homozygous for either the 5HTR1A G allele or the 5HTR2A A allele had lower levels of physical functioning than patients with the other genotypes. These results suggest that 5HTR1A and 5HTR2A promoter variations have gender-dependent modulatory effects on depression and physical function in patients with pain. Furthermore, this study demonstrates that pain after lumbar surgery modulates the association between 5HT gene polymorphisms and depression.

1. Introduction

Major depression and subclinical depressive episodes are important complications in patients with recurrent pain after lumbar disc surgery [15], and pain-related disability is strongly associated with depressive moods [5]. Increasing evidence from clinical and pharmacological studies suggests that dysfunction in the serotonergic (5HT) system plays an important role in the pathophysiology of depression, pain, and disability [41,47,61]. A recent meta-analysis of 54 studies investigating the association between a promoter variant (5HTTLPR) in the 5HT transporter gene and depression yielded strong evidence that this variation, rather than directly influencing depression risk, instead mediates the relationship between stress and depression, particularly when childhood maltreatment, somatic diseases and self-reported somatic complaints were conceptualized as environmental stressors [28].

Although there is evidence that the development of depression is affected by a gene × environment interaction involving the promoter region of the serotonin transporter gene, little is known about the effects of other genes encoding components of the serotonergic system, including the 5HT1A and 5HT2A receptors. A functional promoter polymorphism (–1019C/G, rs6295) in the 5HTR1A gene has been associated with depression [23,36,38,40], anxiety [16,20,55], and antidepressant therapy response [30,37]. Several studies reported higher depression rates in patients...
homzygous for the G allele [34,38,51], while negative findings have also been published [24,49]. However, none of these studies investigated a gene × environment interaction.

The function and regulation of the 5HT2A receptor remain poorly understood, although there are several single nucleotide polymorphisms (SNPs) in this gene that have been investigated, including polymorphisms in the promoter region (−1438A/G, rs6311, and −1027C, rs6313), that are almost in complete linkage disequilibrium [46,58]. Several studies have found evidence for associations between at least one of these 5HT2A SNPs and depression [8,13,14,17,19,25] or antidepressant drug response [12,29]. 5HT2A SNPs were associated with chronic pain diseases such as fibromyalgia, and oral and chronic widespread pain [45,48,57], although studies have reported that these associations were no longer significant after adjusting for depression [48]. Furthermore, few studies have investigated a potential gene × environment interaction for this gene, and those have reported negative findings [19,50].

In addition to the impact of environmental factors, gender also seems to play a modulatory role in the relationship between receptor variation and depression [14,19,31]. However, there is a lack of studies systematically investigating gene × gender interactions in this context.

In the present study, we aimed to examine the impact of 5HT1A (rs6295) and 5HT2A (rs6311) promoter variations on depression in a group of patients who were assessed 6 months after lumbar disc surgery. We hypothesized that the severity of persisting pain may act as a stressor in a gene × environment model to modulate the effect of 5HT1A and 5HT2A variation on depression levels. We further explored the impact of 5HT receptor polymorphisms on the degree of pain-related physical function and disability, and all analyses examined potential interactions with gender.

2. Methods

2.1. Participants

A total of 275 patients who had undergone a first lumbar disc surgery 5 to 9 months previously were recruited at the Department of Neurosurgery of the Knappschaft Hospital at the Ruhr-University of Bochum, Bochum, Germany. All participants were between 18 and 85 years of age and white. Patients were excluded from the study if they had autoimmune arthritis, cancer, a recent fracture, inflammatory disease of the spine, or a previous known psychiatric disease. Thirty-eight participants did not complete the self-reporting questionnaires. A total of 224 participants (114 women and 108 men) participated in the physical examination and were assessed 6 months after lumbar disc surgery 5 to 9 months previously were recruited at the Department of Neurosurgery of the Knappschaft Hospital at the Ruhr-University of Bochum, Bochum, Germany. All participants were between 18 and 85 years of age and white. Patients were excluded from the study if they had autoinflammatory arthritis, cancer, a recent fracture, inflammatory disease of the spine, or a previous known psychiatric disease. Thirty-eight participants did not complete the self-reporting questionnaires. A total of 224 participants (114 women and 108 men) participated in the physical examination and were assessed 6 months after lumbar disc surgery.

Physical functioning and disability were assessed as secondary outcomes. The physical functioning scale (“Funktionsfragebogen Hannover-Rücken”, FFbH-R) is a 12-item questionnaire used to assess pain-related physical functioning in several daily activities (e.g., carrying 10 kg or sitting on a chair for over an hour). Each item can be answered with “yes” (1 point), “yes, narrowly” (2 points), or “no, or with help only” (3 points). The inverted sum score reflects the percentage of physical functioning, where higher scores indicate an increased level of functioning. The FFbH-R has been demonstrated to be a valid and reliable measure of functional status in back pain patients [33]. The Disability Score (DS) is a 3-item component of the Chronic Pain Grade [63] inventory that asks the patient to rate the degree to which pain interferes with functioning in 3 areas of daily living: general daily activities, recreation, and social activities. The mean total DS ranges from 0 to 100, and higher scores indicate higher levels of disability. The German version of the DS was found to be valid and reliable (Cronbach alpha = 0.88) in chronic pain patients [32].

2.2. Measures

2.2.1. Pain intensity

Average pain intensity during the preceding week and average maximum pain intensity during the preceding 3 months were evaluated with an 11-point numeric self-rating scale with the end points 0 (no pain) and 10 (very severe pain). Numeric rating scale pain scores have been demonstrated to be reliable and valid measures in pain research [18,26]. Patients were classified as having no pain when the average pain level during the preceding week was 0, low to moderate pain when the reported pain level was between 1 and 4, and severe pain when the reported pain level was >4.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.69 ± 13.04</td>
</tr>
<tr>
<td>Sex</td>
<td>Female: 116 (51.8%), Male: 108 (48.2%)</td>
</tr>
<tr>
<td>Education</td>
<td>Low (maximum 8 y of schooling): 85 (37.9%), Intermediate (10 y of schooling): 88 (39.3%), High (13 y of schooling plus university in some cases): 51 (22.8%)</td>
</tr>
<tr>
<td>Pain</td>
<td>Average back pain during 7 d, 0–10: 3.06 ± 2.40, Average pain during 3 mo, 0–10: 3.62 ± 2.30, Maximum pain during 3 mo, 0–10: 4.99 ± 2.62</td>
</tr>
<tr>
<td>Beck Depression Inventory</td>
<td>Score, 0–53: 7.16 ± 6.18, Group 0–9: 158 (70.5%), Group 10–18: 50 (22.3%), Group &gt;18: 16 (7.1%)</td>
</tr>
<tr>
<td>FFbH-R, 0–100% (n = 141)</td>
<td>71.07 ± 23.47</td>
</tr>
<tr>
<td>Pain Disability Score, 0–100</td>
<td>32.00 ± 28.08</td>
</tr>
<tr>
<td>5HT2A (rs6295) (n = 221)</td>
<td>CC: 63 (28.5%), CG: 93 (42.1%), GG: 65 (29.4%)</td>
</tr>
<tr>
<td>5HT2A (rs6311) (n = 223)</td>
<td>AA: 43 (19.3%), AG: 96 (43.0%), GG: 84 (37.7%)</td>
</tr>
</tbody>
</table>

FBbH-R, Funktionsfragebogen Hannover-Rücken (physical functioning scale).

2.2.2. Depression

The primary outcome was depression, measured by the Beck Depression Inventory (BDI-I) [6], a 21-item inventory designed to measure responses associated with depression. The current study used the German version introduced by Kammer [27], which has previously exhibited high reliability and validity [53,62].

2.2.3. Self-reported physical functioning and disability in daily life

Physical functioning and disability were assessed as secondary outcomes. The physical functioning scale (“Funktionsfragebogen Hannover-Rücken”, FFbH-R) is a 12-item questionnaire used to assess pain-related physical functioning in several daily activities (e.g., carrying 10 kg or sitting on a chair for over an hour). Each item can be answered with “yes” (1 point), “yes, narrowly” (2 points), or “no, or with help only” (3 points). The inverted sum score reflects the percentage of physical functioning, where higher scores indicate an increased level of functioning. The FFbH-R has been demonstrated to be a valid and reliable measure of functional status in back pain patients [33]. The Disability Score (DS) is a 3-item component of the Chronic Pain Grade [63] inventory that asks the patient to rate the degree to which pain interferes with functioning in 3 areas of daily living: general daily activities, recreation, and social activities. The mean total DS ranges from 0 to 100, and higher scores indicate higher levels of disability. The German version of the DS was found to be valid and reliable (Cronbach alpha = 0.88) in chronic pain patients [32].

2.3. Recruitment and sample collection

Subjects were invited to participate in this study 5 to 9 months after their first lumbar disc surgery. During the initial telephone contact, patients were informed of the study design, screened for inclusion and exclusion criteria, and invited to a physical examination. Individuals willing to participate subsequently received a
questionnaire to fill out. On the day of the physical examination, the completed questionnaires were collected, and a 10-mL EDTA (ethylenediamine tetra-acetic acid) blood sample was taken.

2.4. Genotyping

DNA was extracted according to a standard method [44]. Genotyping for the 2 SNPs in 5HTR1A (rs6295) and 5HTR2A (rs6311) was performed by polymerase chain reaction with subsequent restriction enzyme digestion. The primer sequences consisted of the following: forward, TCT CTC CCG GTT CCC CAA, and reverse, GGA AGA AGA CCG AGT GTG TCA TC for rs6295; and forward, TGC TAA TAG TTT ATC AGA GTT ATC ACC ACA, and reverse, TGC AGA TTC CCA TTA AGG TAG GTA A for rs6311. Polymerase chain reaction products were digested with BseGI (rs6295) and MspI (rs6311). Completion rates were 0.9% and 0.5%, respectively, and probes with ambiguous bands were reanalyzed. Positive and negative controls were included in each analysis.

2.5. Statistical analysis

To investigate the main effects and interactions between genotype, sex, and pain on the depression scores as the primary outcome, we performed 3-way univariate analyses of covariance (ANCOVA) with age as the covariate. Subsequently, we performed 3-way univariate analyses of covariance (ANCOVA) with age as the covariate. In cases of severe pain (>4), women carrying at least one A allele of 5HTR2A rs6311 exhibited a significantly higher BDI score (mean ± standard deviation 14.22 ± 8.27) than women with the GG genotype (7.14 ± 3.82, P = .004, Table 2). In contrast, women who reported no pain and carried at least one A allele revealed a trend toward lower BDI scores (1.83 ± 2.48) compared with GG women (7.56 ± 6.13, P = .051). No effect of genotype was observed for women experiencing a moderate level of pain (pain intensity 1–4) (Fig. 1a). When the depression levels were subdivided into moderate to severe depression (BDI >18), mild depression (BDI 10–18) or no depression (BDI <10), a significant association between depression and the presence of 5HTR2A rs6311 was detected for female patients with moderate to severe pain (χ² = 8.11, P < .05). Women with moderate to high levels of depression all carried at least one A allele of rs6311 (Fig. 1c), whereas male patients only exhibited an effect of pain on depression, which was not influenced by the 5HTR2A genotype (Fig. 1b). We found no main effect of genotype on intensity of pain.

Because of the small sample size of the subgroup of 5HTR1A GG women with no pain (n = 1), we decided to evaluate 5HTR1A only for the effects of genotype and sex, without including the pain factor. With respect to physical function and disability, 2-way ANCOVAs were performed with age, pain intensity, and depression used as covariates to explore the main effects of genotype and sex as well as potential interactions. Because no coherent reports on the respective alleles or genotypes that convey risks of low physical functioning or disability were available from the literature, we tested all 3 genotype groups separately for this question. The level of significance was set at P < .05 for all comparisons. Because of multiple testing for 2 genes, this level was adjusted to P < .025. The analyses were performed by SPSS software, version 19.0 (IBM, Armonk, NY).

3. Results

3.1. Sample characteristics

Table 1 lists descriptive data on sociodemographic variables, genotype distributions, pain intensity, and the primary and secondary outcomes depression, physical function, and disability in daily life. The mean age of the patients was 49.7 years, and 52% were women. The mean ± standard deviation pain intensity score for the previous 7 days was 3.06 ± 2.40, and the mean depression score was 7.16 ± 6.18. Thirty-eight patients (17.0%) reported no pain, 118 (52.7%) reported low to moderate pain, and 68 (30.4%) reported severe pain. According to the BDI scale, 158 patients (70.5%) had no signs of depression, 50 (22.3%) had mild clinical symptoms, and 16 (7.1%) had moderate to severe levels of depressive mood.

In this cohort, the genotypes for rs6311 were in Hardy-Weinberg equilibrium (P = .127), whereas a slight deficiency of heterozygotes was observed for rs6295 (P = .022). Genotyping errors were excluded by random repeat evaluation.

3.2. Depression

The BDI scores stratified according to 5HTR1A (GG vs CG/CC) and 5HTR2A (AA/AG vs GG) genotype, sex, and the 3 levels of pain (no pain, pain intensity 1–4, and pain >4) are presented in Table 2. For 5HTR2A, a 3-way analysis of covariance for the 5HTR2A genotype, sex, and the 3 levels of pain as the between factors and age as the covariate revealed a significant gene × sex × pain interaction (F = 5.489, P = .005, Table 2). In cases of severe pain (>4), women carrying at least one A allele of 5HTR2A rs6311 exhibited a significantly higher BDI score (mean ± standard deviation 14.22 ± 8.27) than women with the GG genotype (7.14 ± 3.82, P = .004, Fig. 1a). In contrast, women who reported no pain and carried at least one A allele revealed a trend toward lower BDI scores (1.83 ± 2.48) compared with GG women (7.56 ± 6.13, P = .051). No effect of genotype was observed for women experiencing a moderate level of pain (pain intensity 1–4) (Fig. 1a). When the depression levels were subdivided into moderate to severe depression (BDI >18), mild depression (BDI 10–18) or no depression (BDI <10), a significant association between depression and the presence of 5HTR2A rs6311 was detected for female patients with moderate to severe pain (χ² = 8.11, P < .05). Women with moderate to high levels of depression all carried at least one A allele of rs6311 (Fig. 1c), whereas male patients only exhibited an effect of pain on depression, which was not influenced by the 5HTR2A genotype (Fig. 1b). We found no main effect of genotype on intensity of pain.

Because of the small sample size of the subgroup of 5HTR1A GG women with no pain (n = 1), we decided to evaluate 5HTR1A only for the effects of genotype and sex, without including the pain factor. This analysis revealed a borderline significant main effect for 5HTR1A genotype on depression; patients homozygous for the 5HTR1A G allele had higher BDI scores relative to the CC/CG group (F = 3.909, P = .049, Table 2). No gender effect was identified for 5HTR1A variation and depression scores. We detected no main effect of genotype on pain intensity.

3.3. Physical function and pain-related disability in daily life

Table 3 presents the scores for physical function and disability in daily life stratified by 5HTR1A (n = 139) and 5HTR2A (n = 141) genotype and sex. In regard to physical function, a 2-way analysis of covariance with 5HTR1A genotype and sex as the between factors and age, pain intensity, and depression as covariates revealed a significant gene × sex interaction (F = 3.303, P = .040, Table 3). A subsequent ANCOVA including only female subjects revealed a significant main association between 5HTR1A genotype and lower physical function in women harboring the GG genotype compared to the CC/CG genotype (F = 4.945, P = .030). Men did not exhibit any differences related to genotype (Fig. 2a).

For 5HTR2A, a 2-way analysis of covariance with genotype and sex as the between factors and age, pain intensity, and depression as covariates revealed significant main effects for genotype (F = 4.279, P = .016) and sex (F = 11.942, P = .001) as well as a significant genotype × sex interaction (F = 5.285, P = .006, Table 3). A subsequent ANCOVA including only female subjects indicated a significant main effect for 5HTR2A genotype (F = 7.089, P = .002). Bonferroni post hoc tests revealed significantly lower physical functioning scores in women with the AA genotype compared to the AG and GG genotypes (P ≤ .002), whereas men did not exhibit significant differences between genotypes (Fig. 2b).

Concerning disability in daily life, a 2-way analysis of covariance that was calculated with 5HTR1A genotype and sex as the between factors and age, pain intensity, and depression as covariates revealed a significant gene × sex interaction (F = 4.355, P = .014, Table 3). Two subsequent ANCOVAs that were performed for men and women separately indicated a trend toward a genotype
### Table 2

Results of analyses of covariance with age as covariate of BDI depression scores stratified for 5HTR1A and 5HTR2A risk genotypes, levels of pain, and sex.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male (N)</th>
<th>Female (N)</th>
<th>Mean ± Standard Deviation</th>
<th>F(df1,df2)</th>
<th>P</th>
<th>G × E</th>
<th>G × S</th>
<th>G × E × S</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HTR1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>7</td>
<td>2</td>
<td>3.29 ± 6.55</td>
<td>1</td>
<td>.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG/CC</td>
<td>14</td>
<td>14</td>
<td>3.67 ± 2.78</td>
<td>14</td>
<td>.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA/AG</td>
<td>17</td>
<td>6</td>
<td>3.41 ± 4.48</td>
<td>14</td>
<td>.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5HTR2A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AA/AG</td>
<td>17</td>
<td>6</td>
<td>3.41 ± 4.48</td>
<td>14</td>
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<tr>
<td>GG</td>
<td>7</td>
<td>2</td>
<td>3.29 ± 6.55</td>
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<tr>
<td>CG/CC</td>
<td>14</td>
<td>14</td>
<td>3.67 ± 2.78</td>
<td>14</td>
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<tr>
<td>AA/AG</td>
<td>17</td>
<td>6</td>
<td>3.41 ± 4.48</td>
<td>14</td>
<td>.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± standard deviation.*

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**Results**

- **5HTR1A:**
  - No significant effect observed for the GG genotype.
  - Significant effect observed for the CG/CC genotype (P = .045, F = 3.909, P < .05).

- **5HTR2A:**
  - Significant effect observed for the AA/AG genotype (P = .047, F = 2.964, 0.01 < .05).

**Discussion**

To our knowledge, this is the first study to report the main effects of genetic variation in the serotonin system as well as gene × environment interaction effects on depression in patients 6 months after lumbar disc surgery. Three levels of pain (no pain, mild to moderate pain, severe pain) served as a disease-related distress variable in this analysis.

We found, as expected, strong evidence for a gene × environment × sex interaction for the 5HTR2A promoter SNP, as female patients carrying at least one A allele of the −1438A/G promoter SNP (rs6311) reported significantly higher depression scores when confronted with severe pain than did women with the GG genotype. In contrast, when free of pain, women with the AA/AG genotype had lower levels of depression than women with the GG genotype. Men did not demonstrate any effect of the rs6311 genotype on depression rates.

These findings are in accordance with previous results concerning variations in other genes encoding components of the 5HT system. A recent meta-analysis of a promoter variant (5HTTLPR) in the 5HT transporter gene in association with depression found strong evidence that this variant, rather than directly influencing depression risk, modulates the relationship between stress and depression via gene × environment interaction effects [28]. These effects were predominantly observed in clinical subgroups demonstrating childhood maltreatment, severe diseases, and self-reported physical complaints as environmental stressors [28]. Our results indicate that pain after lumbar disc surgery may be interpreted as such stressor that can modulate the effects of genotype on depression. This is in line with Max et al. [42], who defined lumbar spine, nerve root, and its pain input to the central nervous system as part of the environment in which the brain generates an affective state. As a stressor, pain stimulates a wide variety of psychological and physiological responses, including the activation of the hypothalamic–pituitary–adrenal axis [59], which is involved in complex interrelations with the 5HT system [52]. The results of the present study also support the hypothesis that not only early life stress but also recently experienced stressors may be involved in complex gene × environment interactions and that the 5HTR2A promoter SNP may represent an additional physiologically relevant polymorphism within the 5HT neurotransmitter system [11].

With respect to a direct impact of 5HTR2A variation on depression, previous studies have yielded inconsistent results. Several studies revealed a main effect of 5HTR2A variation on depression [8,25], but certain studies only observed this effect in men [25], whereas others detected it only in women [39]. These results are in accordance with our finding of a gender-dependent gene × environment interaction for the 5HTR2A promoter SNP. Gender differences in the 5HT system have been previously reported [19,25], although the mechanisms underlying these phenomena remain unclear [9]. Female subjects are generally more sensitive to the onset of pain, as they subsequently demonstrate higher depression scores and lower levels of physical functioning [1,35,56]. This gender difference may be related to hormonal status, as progesterone and estrogen are known to modulate 5HT reuptake [43]. Furthermore, the different pain coping strategies used by men and women are well established and may help to explain our findings for the 5HTR2A SNP [1,54].

We identified a borderline main effect on depression for the functional promoter SNP −1019C/G in the 5HTR1A gene, with
patients homozygous for the G allele having higher BDI scores than patients with CG/CC genotypes. This finding is in accordance with a previous study reporting an association of the G allele with increased depressive symptoms after hip fracture [39]. A main effect of the −1019 C/G polymorphism in the 5HTR1A gene without a gene × environment interaction was also recently demonstrated to predict cortisol stress responses in healthy individuals [4].

In addition to depression, we also analyzed the 5HTR1A and 5HTR2A promoter polymorphisms in relation to physical functioning and pain-related disability, which are clinical variables that have not been thoroughly investigated. We found significant associations of both polymorphisms with physical functioning, although these effects were again restricted to female patients. Women homozygous for the 5HTR1A G allele or the 5HTR2A A allele had significantly lower physical functioning rates 6 months after lumbar disc surgery than did women of the other 2 genotypes. These findings remained significant after controlling for age, depression, and pain intensity. Furthermore, we found higher pain-related disability scores in male subjects homozygous for the 5HTR1A G allele, while no association was observed for the 5HTR2A promoter SNP. The relationship between variation in the 5HT gene system and disability remains poorly understood; one study reported an association of 5HTR1A variation with poor functional recovery after hip surgery [39], but further research is clearly needed. 5HT1A/5HT2A receptors are present in cortical areas, motoneurons and muscle cells, and analyses of Parkinson’s patients experiencing levodopa-induced dyskinesia [10] have suggested that these receptors may suppress motor function, which may explain how they influence disability. As there is substantial evidence that cognitive/behavioral factors play a significant role

Table 3

Results of analyses of covariance with age, pain, and depression as covariates for physical function (n = 141) and disability scores (n = 217) stratified for 5HTR1A and 5HTR2A genotypes and sex.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Physical function</th>
<th>G</th>
<th>G × S</th>
<th>Disability</th>
<th>G</th>
<th>G × S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Fdf₂</td>
<td>Fdf₂</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>5HTR1A</td>
<td></td>
<td>N</td>
<td>MW ± SD</td>
<td>N</td>
<td>MW ± SD</td>
<td>N</td>
</tr>
<tr>
<td>GG</td>
<td>18</td>
<td>72.92 ± 21.26</td>
<td>21</td>
<td>56.54 ± 26.04</td>
<td>1.455</td>
<td>3.303*</td>
</tr>
<tr>
<td>CG</td>
<td>33</td>
<td>82.32 ± 16.44</td>
<td>27</td>
<td>70.83 ± 23.76</td>
<td>Female:</td>
<td>38</td>
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<tr>
<td>CC</td>
<td>25</td>
<td>67.83 ± 23.69</td>
<td>15</td>
<td>66.67 ± 24.39</td>
<td>GG &lt; CG = CC</td>
<td>32</td>
</tr>
<tr>
<td>5HTR2A</td>
<td></td>
<td>N</td>
<td>MW ± SD</td>
<td>N</td>
<td>MW ± SD</td>
<td>N</td>
</tr>
<tr>
<td>AA</td>
<td>18</td>
<td>81.94 ± 14.64</td>
<td>8</td>
<td>44.79 ± 25.27</td>
<td>4.279*</td>
<td>5.285**</td>
</tr>
<tr>
<td>AG</td>
<td>29</td>
<td>73.99 ± 21.92</td>
<td>31</td>
<td>70.83 ± 25.04</td>
<td>Female:</td>
<td>40</td>
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<td>25</td>
<td>65.66 ± 22.70</td>
<td>AA &lt; AG = GG</td>
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</tr>
</tbody>
</table>

* P < .05
** P < .01.

Fig. 1. Comparison of BDI depression scores (mean, SEM) for women (a) and men (b) with at least one A allele (AA/AG) of the 5HTR2A promoter polymorphism compared to women with the GG genotype, stratified by the 3 levels of reported pain. (c) Within the subgroup of women with pain intensity >4 (n = 32), the percentage of women with at least one A allele (AA/AG) is shown relative to that of women with the GG genotype at the 3 levels of depression.
in the development of disability and depression in pain patients [22,23], future studies should evaluate these potential gene × behavior interactions in greater detail.

We did not hypothesize a main effect of serotonin receptor genes on pain, although several candidate gene studies have demonstrated associations with chronic pain diseases such as fibromyalgia [7], temporomandibular pain [57], and chronic widespread pain [48]. Because depression is mostly increased in these chronic diseases, it cannot be ruled out reliably that the gene–pain relation was mediated by depression. The well-controlled population-based study by Nicholl et al. [48], which used a discovery and a validation cohort, failed to demonstrate a main effect on pain after adjusting for depression. In a post hoc analysis of our cohort, we did not detect a main effect of genotype on pain.

The present study had several limitations. First, because we could not examine the same patients before lumbar disc surgery, we do not have baseline depression and disability scores. However, a recent prospective study in surgically treated patients with low back pain revealed a significant gene × environment interaction on mood controlling for baseline depression [42]. Second, the measure of depression used was a self-reporting questionnaire rather than diagnostic interviews, which would most likely have delivered a more valid classification. A strength of this measurement, on the other hand, is a high reliability producing low measurement error. This is important because the sample size of this study is, as a result of a restricted budget, relatively small [65]. Nevertheless, the highly significant gene × environment interaction in the female sample found in this study needs to replicate in a larger sample. Furthermore, we did not ask the subjects about their use of antidepressant medications, so there may be a bias in the reported depression rates. However, because the analyses were performed in a homogeneous sample of white patients who had all undergone lumbar disc surgery performed in a consistent operating method at the same hospital, we think that we have analyzed an appropriate and representative population of chronic back pain patients. Finally, we examined only 1 SNP each for 5HTR1A and 5HTR2A instead of comprehensively covering greater genetic variation in these genes, eg, with a full haplotype analysis. We chose to concentrate on the promoter regions of these genes because there is evidence for stress-dependent binding of transcription factors and methylation [21]. The −1438A/G promoter polymorphism in 5HTR2A is in almost complete linkage disequilibrium with another promoter SNP (–1027T/C, rs6313) that has been analyzed in several previous studies [46,58], so the analysis of this variant would not have provided additional information. We acknowledge that linked variation within the 5HTR1A and 5HTR2A haplotypes may be responsible for the observed association results, but evidence is accumulating that functional variation in these 2 genes contributes to the development of pain and depression in complex ways.

In conclusion, this study revealed evidence for an impact of 5HTR1A and 5HTR2A promoter variation on depression, physical function, and disability as well as complex interaction effects between environmental stressors and gender in patients 6 months after lumbar disc surgery. To elucidate the influence of genetic markers of the serotonergic system on depression and related variables in pain patients, replication studies in larger patient samples and challenging new research designs to test interactions between multiple functional genetic variants and environmental influences [64] seem warranted.

Conflict of interest statement

The authors report no conflict of interest.

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References


