

## 5-HTTLPR polymorphism of serotonin transporter and effects of sertraline in terminally ill cancer patients: report of eleven cases

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### ABSTRACT

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Depression is difficult to detect in cancer patients, though its determination offers an opportunity to relieve patients' suffering in palliative care. Selective serotonin reuptake inhibitors (SSRIs) are the treatment of choice for mood disorders, but they show a highly variable response. The short allelic variants "s/s" and "s/l" of the 5-HTTLPR polymorphism in the promoter region of the serotonin transporter gene has been consistently associated with a poorer response to SSRIs. The aim of this study has therefore been to examine depression, anxiety and mental adaptation to cancer in terminally ill and depressed cancer patients, in relation to treatment with sertraline and to the 5-HTTLPR genetic polymorphism. Eleven consecutive depressed patients with different forms of advanced cancer who were admitted to the Hospice of the Casa di Cura "Pineta del Carso" (Trieste, Italy) were treated with sertraline for two weeks and their response was determined and related to 5-HTTLPR.

Sertraline significantly reduced the average depression and anxiety subscale scores of HADS, as well as the scores of the subscales of Mini-MAC. When the effects of sertraline were analyzed in relation to the 5-HTTLPR polymorphism, only patients with the "l/l" allelic variant had significantly lower scores of HADS anxiety, Mini-MAC hopelessness-helplessness and anxious preoccupation, and a higher score for the fighting spirit of Mini-MAC; the depression score was significantly reduced in patients with both allelic variants. These data indicate that sertraline is effective after two weeks of treatment in terminally ill cancer patients, acting not only on depression but also on anxiety and mental adaptation to cancer. Moreover, the effect of sertraline significantly depended on the genetic polymorphism of the serotonin transporter, being more pronounced in patients carrying the "l/l" genetic variant; these findings seem to encourage the examination of a larger sample of patients.

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### Introduction

Palliative care of terminally ill cancer patients frequently involves the administration of analgesic agents, as well as psychotropic drugs such as anxiolytics, antipsychotics and antidepressants<sup>1,2</sup>. Depression and other mood disorders occur frequently in cancer patients<sup>3-5</sup> and depressive symptoms may be particularly difficult to detect in patients with terminal illness<sup>6,7</sup>. As a result, depression is frequently not diagnosed and not treated, with negative consequences for the quality of life of the patients<sup>8</sup>. The identification of patients suffering from depression thus might provide clinicians with an opportunity to considerably relieve patients' suffering<sup>9</sup>. Selective serotonin reuptake inhibitors (SSRIs) are currently the standard choice of antidepressant agents. However, the response to antidepressants displays remarkable heterogeneity, attributed to a variety of possible causes including the genetic polymorphism of the patients<sup>10,11</sup>. The serotonin-transporter-linked polymorphic region (5-HTTL-

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PR) is a polymorphism of the promoter region of the serotonin transporter gene, consisting of an insertion/deletion of 44 bp giving a long ("l") or short ("s") allele<sup>12</sup>. The short allelic variants "s/s" and "s/l" of 5-HTTLPR have been consistently associated with a poorer response to treatment with various SSRIs and with a slower improvement of depressive "core" and somatic anxiety symptoms than that of "l/l" carriers<sup>13-17</sup>.

The aim of this study has therefore been to examine a series of terminally ill cancer patients admitted to the Hospice of the Casa di Cura "Pineta del Carso" who had received a diagnosis of depression. They were treated with sertraline as an SSRI antidepressant, and their response to this treatment was analyzed in relation to the genetic polymorphism 5-HTTLPR of the serotonin transporter (SERT). The patients were evaluated for anxiety and depression using the HADS scale<sup>18</sup>, and for mental adjustment to cancer using the Mini-MAC scale<sup>19</sup>; such evaluation took place at admission and recruitment into the study, and after treatment with sertraline. The results obtained by analyzing these variables and their interactions in terms of differences in treatment outcome and associations between drug response and genetic polymorphism are reported here.

## Patients and methods

### Patients

The study cohort comprised 23 consecutive patients, 14 men (61%) and 9 women (39%) admitted to the Hospice of the Casa di Cura "Pineta del Carso" (Trieste, Italy) between October 2005 and June 2006, who met the criteria for depressive disorder as determined with a structured clinical interview according to the DSM-IV. All patients had received a diagnosis of cancer, and their tumors were in the lung (5 patients), bowel (3 patients), stomach (3 patients), kidney (2 patients), prostate, brain, duodenum, breast, submandibular gland, biliary duct, and bronchus in 1 patient each; a plasmocytoma and 2 tumors of unknown origin were present in 3 patients. Nine of the patients (39%) had been treated with chemotherapy, 4 (17%) with radiotherapy, and 12 (52%) with surgery; 16 patients (70%) had metastases.

### Treatment and psychometry

The patients were receiving treatment with one or more drugs: 11 patients (48%) received non-opioid analgesics for pain, 10 (43%) opioids, 21 (91%) benzodiazepines and 4 (17%) received antipsychotics. The majority (78%) had a Karnofsky performance score of 40-60.

The study patients were characterized psychometrically for depression and anxiety by a trained psychologist using the Hospital Anxiety and Depression Scale (HADS)<sup>18</sup>, and for their mental adaptation to cancer us-

ing the Mini-Mental Adjustment to Cancer Scale (Mini-MAC)<sup>19</sup>. The psychometric scales were administered at admission to the hospice and recruitment to the study (T0), and after daily treatment with oral sertraline at a dose of 50 mg for 14 days (T1). During the 2-week period of observation, 4 patients were discharged and 8 died: 11 patients thus completed both assessments at T0 and T1.

The patients received detailed information about the study procedure and expressed their written informed consent to participate in the investigation, which was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. For each patient, demographic as well as previous and current medical history were recorded anonymously for later analysis.

### Polymorphism analysis

The patients were also genotyped for 5-HTTLPR evaluation by analysis of genomic DNA obtained from whole blood or buccal cells using standard procedures (MasterAmp<sup>TM</sup> buccal swab brushes, Epicentre Biotechnologies; GenElute<sup>TM</sup> Blood Genomic DNA Kit, Sigma). Polymerase chain reaction (PCR) amplification of 5-HTTLPR was performed using the primers described by Gelernter<sup>20</sup>, and with the GC-Rich PCR System (Roche Molecular Biomedicals) in a 50- $\mu$ L reaction containing 20-100 ng of DNA; 100- $\mu$ M deoxyribonucleoside triphosphate (dNTPs), 20 pmol for each primer, and 1.5 mM MgCl<sub>2</sub>. DNA was denatured at 95 °C for 10 minutes and subjected to 40 cycles of 40 seconds of denaturation at 94 °C, 45 seconds of annealing at 56 °C, 40 seconds of extension at 72 °C, and 10 minutes of final extension at 72 °C. The products of PCR amplification were separated on a 2% agarose gel, and were visualized in ultraviolet light after ethidium bromide staining.

### Statistical analysis

The scores of the psychometric scales obtained at admission (T0) and after 14 days of treatment (T1) were analyzed in relation to the genotypes of the patients using nonparametric techniques. For the patients who could be tested after 14 days of sertraline administration, the scores of the psychometric scales were analyzed again in relation to drug treatment and genotypic characterization. Standard techniques including the Kruskal-Wallis, Mann-Whitney and Wilcoxon tests, as well as chi-square analysis for multiway frequency tables, were employed using the standard software package Systat 10 (Systat Software Inc., San Jose, California, USA).

## Results

Analysis of the subscale scores of the psychometric assessment revealed no significant differences at ad-

mission between patients who dropped out and those who completed the study in any variable considered including sex, previous treatment, tumor stage, Karnofsky status, opioid use, HADS and Mini-MAC scores, and genotype polymorphisms (Fisher's exact test and Kruskal-Wallis test).

Treatment with sertraline for 2 weeks significantly reduced the scores for anxiety and depression of the HADS scale, decreased the scores for hopelessness-helplessness, anxious preoccupation and fatalism, and increased the score of avoidance of the Mini-MAC subscales (Table 1).

Of the 11 patients remaining at T1, 1 patient (9%) was a carrier of the "s/s" allelic variant according to 5-HTTLPR genotype analysis, 4 (36%) of the "s/l" allele and 6 (54%) of the "l/l" allele. No significant association was found between the scores of the psychometric scales and the clinical and demographic variables (sex, cancer stage, Karnofsky status, 5-HTTLPR genotype; Wilcoxon and Kruskal-Wallis tests). When the effects of treatment with sertraline were analyzed in relation to the polymorphism considered, HADS anxiety, Mini-MAC hopelessness-helplessness and anxious preoccupation scores were significantly decreased only in patients with the "l/l" allelic variant of SERT, and the fighting spirit score of Mini-MAC was similarly increased in these patients. The HADS depression score decreased in patients with both types of allelic variant (Table 2).

## Discussion

The results obtained by analyzing the patients at admission and inclusion in the study indicate that anxiety

and depression identified using HADS, as well as the modalities of mental adaptation to cancer determined using Mini-MAC, did not appear to depend on the gender of the patients, on the presence of systemic disease, on the performance status of the patients, on the different treatments received, and on the allelic variants of 5-HTTLPR polymorphism. Likewise, treatment for 2 weeks with psychotropic and analgesic drugs did not have any statistically significant effect on HADS and Mini-MAC scales. On the other hand, treatment with sertraline for 2 weeks resulted in a significant reduction in the depression and anxiety subscales of HADS, and in hopelessness-helplessness, anxious preoccupation and fatalism of Mini-MAC.

Molecular genetic characterization of 5-HTTLPR in SERT indicates that approximately the same proportion of patients had the "l/l" (6/11) and the "s/s" and "s/l" (total of 5/11) allelic variants, which were shown to cause high ("l/l") or low ("s/s", "s/l") functional activity of SERT, respectively<sup>12</sup>. When the effects of sertraline were analyzed in relation to the SERT genotype, a significant reduction on the HADS anxiety, Mini-MAC hopelessness-helplessness and anxious preoccupation scores, as well as an increase of the fighting spirit score of Mini MAC were observed only for the "l/l" variant with high functionality. The effects of sertraline on depression that were generally observed in the whole patient cohort occurred in both groups of allelic variants.

The data presented on the effects of sertraline indicate a significant response in terminally ill cancer patients of both genders, which is not limited to depression and which is established already after 2 weeks of treatment. Moreover, in agreement with the available evidence, they show a better response for patients car-

**Table 1 - Effects of treatment with sertraline**

	No.	Time (days°)	Average score (± SE)	Median (quartiles)	P*
HADS Depression	11	0	11.3 ± 0.9	12.0 (9.0-12.0-14.0)	0.003
		14	6.7 ± 1.0	6.0 (4.0-6.0-8.0)	
HADS Anxiety	11	0	6.7 ± 1.3	5.0 (3.0-5.0-12.0)	0.020
		14	3.5 ± 0.9	3.0 (1.0-3.0-5.0)	
Mini-MAC Hopelessness-helplessness	11	0	20.4 ± 0.6	21.0 (18.0-21.0-22.0)	0.017
		14	17.0 ± 0.9	17.0 (16.0-17.0-20.0)	
Mini-MAC Anxious preoccupation	11	0	17.6 ± 0.9	17.0 (15.0-17.0-21.0)	0.007
		14	14.6 ± 0.8	15.0 (12.0-15.0-16.0)	
Mini-MAC Fatalism	11	0	12.5 ± 0.4	13.0 (12.0-13.0-13.0)	0.040
		14	11.6 ± 0.4	11.0 (11.0-11.0-13.0)	
Mini-MAC Avoidance	11	0	9.8 ± 1.3	8.0 (5.0-8.0-16.0)	0.035
		14	10.8 ± 0.7	12.0 (9.0-12.0-13.0)	

SE, standard error.

°The scores reported are those at admission (T0) and at retest 14 days later (T1).

\*Probability, Wilcoxon test.

**Table 2 - Response to treatment with sertraline and SERT genotype**

	Allelic variant (functionality)	No.	Time (days <sup>o</sup> )	Average score $\pm$ SE	Median (quartiles)	P*
HADS Anxiety	s/s, s/l (low)	5	0	6.0 $\pm$ 2.3	4.0 (1.5-4.0-11.5)	0.279
			14	3.2 $\pm$ 0.8	3.0 (1.5-3.0-5.0)	
	l/l (high)	6	0	7.3 $\pm$ 1.7	7.0 (3.5-7.0-12.0)	0.046
			14	3.8 $\pm$ 1.7	2.5 (0.0-2.5-8.5)	
HADS Depression	s/s, s/l (low)	5	0	10.8 $\pm$ 1.8	9.0 (7.5-9.0-13.5)	0.034
			14	8.0 $\pm$ 2.2	7.0 (3.5-7.0-13.0)	
	l/l (high)	6	0	11.7 $\pm$ 1.0	12.0 (9.0-12.0-13.5)	0.027
			14	5.7 $\pm$ 0.8	6.0 (5.0-6.0-6.5)	
Mini-MAC Hoplessness-helplessness	s/s, s/l (low)	5	0	20.5 $\pm$ 0.9	22.0 (18.5-22.0-22.0)	0.285
			14	17.8 $\pm$ 1.6	18.0 (14.5-18.0-21.5)	
	l/l (high)	6	0	20.3 $\pm$ 0.8	20.5 (18.0-20.5-22.3)	0.043
			14	16.3 $\pm$ 1.2	16.5 (14.8-16.5-18.5)	
Mini-MAC Fighting spirit	s/s, s/l (low)	5	0	14.4 $\pm$ 0.6	15.0 (13.0-15.0-15.5)	0.102
			14	12.6 $\pm$ 1.4	13.0 (9.5-13.0-15.5)	
	l/l (high)	6	0	13.0 $\pm$ 1.1	12.5 (10.8-12.5-15.5)	0.041
			14	15.5 $\pm$ 0.6	15.5 (14.0-15.5-17.0)	
Mini-MAC Anxious preoccupation	s/s, s/l (low)	5	0	17.6 $\pm$ 1.9	19.0 (13.0-17.0-21.5)	0.066
			14	14.6 $\pm$ 1.4	15.0 (12.0-15.0-17.0)	
	l/l (high)	6	0	17.7 $\pm$ 1.0	17.0 (15.8-17.0-20.3)	0.039
			14	14.7 $\pm$ 1.0	16.0 (11.8-16.0-16.3)	

SE, standard error.

<sup>o</sup>The scores reported are those at admission (T0) and at retest 14 days later (T1).

\*Probability, Wilcoxon test.

rying the "l/l" variant of the SERT genotype with high functional activity. These results are in agreement with the pharmacogenetics of antidepressant drugs in general<sup>13-17</sup>.

Our findings appear to be relevant for palliative care because depression was shown to lower the pain threshold (amplifying the pain perceived)<sup>21</sup>, to lower the compliance to treatment<sup>22</sup>, and to be related to a shorter life expectancy<sup>23</sup>, to maladaptive styles of coping with the disease<sup>24</sup>, to abnormal illness behavior (dysphoria and irritability)<sup>5</sup> and to suicidal risk<sup>25,26</sup>. Pharmacotherapy with antidepressants thus may considerably relieve patients' suffering and allay distress in the terminal stage of illness, improving coping skills and quality of life<sup>27</sup>.

The results reported indicate that the effects of sertraline appear to depend on pharmacogenetics also in terminally ill cancer patients, and that the observed effects are exerted only in those patients who carry the responsive allelic variant of the SERT polymorphism. The use of SSRIs might thus be guided by molecular genetic analysis also in terminally ill cancer patients. Further research with a larger group of patients, which is partially in progress, may confirm these results, may allow the analysis of possible interactions between the different

variables considered, and may clarify the role that sertraline and SSRIs may have in palliative care.

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