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Mental Adaptation to Mammary Cancer and VNTR Genetic Polymorphism of MAO-A

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ABSTRACT: In women with early breast cancer, the carriers of the genetic polymorphism variants of serotonin transporter (5-HTTLPR) conferring low functional activity displayed high anxious preoccupation as mental adaptation to cancer (MAC) at follow-up. Therefore, we examined the role of the genetic polymorphism VNTR of monoamine oxidase-A (MAO-A) in the same patients.

Mental adaptation to cancer was assessed using Mini-MAC scales at recruitment and at follow-up after 3 months. VNTR polymorphism of MAO-A was determined from blood or oral mucosa samples using conventional laboratory procedures.

Women with the low-functionality activity MAO-A variant displayed significantly less anxious preoccupation at follow-up, as compared to the highly functional ones. No statistically significant effects of VNTR genotype and time were observed for the other subscales of Mini-MAC.

The genotyping of women with early breast cancer for MAO-A VNTR, in addition and together to that for 5-HTTLPR, may allow the identification of the subjects that are likely to display a reduced reduction of their initial anxious preoccupation at follow-up. Interventions may be thus be aimed at the subjects in greater need of support. In the case of drug therapy, these results may allow the choice of the drug with the most appropriate mechanism of action and pharmacogenetic properties.

KEY WORDS: Early breast cancer, mental adaptation to cancer, anxious preoccupation, genetic polymorphism, monoamine oxidase-A

I. INTRODUCTION

The communication of the diagnosis of a malignant tumor constitutes a remarkable stressful life event for the patient and requires adequate mental adaptation, particularly in the early phases of treatment, which are characterized by choices among different therapeutic options and by the acceptance of the therapies themselves. Mental adaptation to cancer has been characterized by specific mechanisms, among which depression and hopelessness-helplessness have been identified in patients with breast cancer and have been shown to be significant negative prognostic factors for survival (Watson et al., 1999; DiMatteo et al., 2000; Walker et al., 1999; Weihs et al., 2000; Hjerl et al., 2003).

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Serotonergic neurotransmission is a central mechanism governing psychological brain functions, and its disregulation has been associated with psychiatric disorders and has been the focus of the mechanism of action of psychiatric drugs (Owens and Nemeroff, 1994; Ressler and Nemeroff, 2000; Schloss and Williams, 1998; Luddington et al., 2009). An initial observation by Caspi et al. showed that a functional genetic polymorphism of serotonin transporter (SERT), called 5-HTTLPR, (5-hydroxytryptamine transporter gene-linked polymorphic region) and consisting in the insertion-deletion of a 44 bp in the promoter region of SERT, modulates the influence of stressful life events by causing depressive conditions via a gene–environment interaction (Caspi et al., 2003). These initial findings have been followed and expanded by an extensive series of experimental studies recently reviewed by Caspi et al. (2010).

The role of the polymorphism of SERT has been also investigated in relation to mental adaptation to cancer in women with early breast cancer. The patients were characterized psychometrically after the communication of the diagnosis using the Mini-Mental Adjustment to Cancer Scale (Mini-MAC) at recruitment and at follow-up, and these patients were genotyped for 5-HTTLPR. Mental adaptation to the disease was found to be associated with high scores of avoidance and anxious preoccupation, which spontaneously decreased with time at follow-up. A decrease of anxious preoccupation was significantly less pronounced in patients with the S/S and S/L genetic variant of 5-HTTLPR compared with the L/L carriers (Schillani et al., 2012).

The synaptic availability of serotonin depends on several factors (Belmaker, 2008), which may act in combination with the function of serotonin transporter already mentioned and the enzymatic degradation of monoamines in the presynaptic terminal, appear to play a significant role. A functional variable number tandem repeat (VNTR) polymorphism has been identified in the promoter gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (MAO-A), existing in 3, 3.5, 4, or 5 repetitions of a sequence of 30 bp (Deckert et al., 1999) and conferring remarkably different functionalities to the enzyme (Sabol et al., 1998). In addition, Caspi et al. showed that maltreated children with the genotype conferring high levels of MAO-A expression were less likely to develop antisocial problems, demonstrating a gene by enviromental interaction (Caspi et al., 2002).

Therefore, we considered the role of VNTR polymorphism of MAO-A in the mental adaptation to cancer and in relation to 5-HTTLPR polymorphism. The samples from patients with early breast cancer, which were initially examined for 5-HTTLPR, were reanalyzed for VNTR of MAO-A, and the data obtained were related to scores of the Mini-MAC subscales at recruitment and follow-up. These results are reported hereafter.

II. MATERIALS AND METHODS

A Subjects and Psychological Measures

The study population comprised a sample of 53 women who received a diagnosis of mammary carcinoma and were referred to the Centro Sociale Oncologico, Azienda Servizi Sanitari 1, Trieste, Italy, between February 2008 and August 2009. The patients were recruited after communication of the cancer diagnosis and surgery and before the beginning of adjuvant treatment (average time 135 ± 9.7 days). The women were evaluated at enrollment into the study (T0) and 3 months later (T1), using the Mini-Mental Adjustment to Cancer Scale (Mini-MAC) (Grassi et al., 2004, 2005) to examine the mechanisms of psychological adaptation to the disease.

The sociodemographic characteristics of patients are described in the article of Schillani et al. (2012) Subjects older than 75 years, and those with a previous or current history of psychiatric disorder, were excluded from the study.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines after it was approved by the relevant institutional Ethical Committee and after informed consent was received from each participant.

B Genotyping

Genomic DNA was obtained from whole blood or buccal cells, using standard procedures (Master Amp^{TM} buccal swab brushes, Epicentre Technologies; GenElute^{TM} blood Genomic DNA Kit, Sigma).

The genotype of MAO-A VNTR polymorphism was determined using the primers described by Sabol et al. (1998) and the GC-Rich PCR system (Roche Molecular Biomedicals) in a 50 μL reaction containing 20–100 ng of DNA; 100 μm deoxyribonucleoside triphosphate (dNTPs), 20 pmol for each primer, and 1.5 mM $MgCl_2$; The DNA was denatured at 95°C for 7 min and subjected to 40 cycles of 40 s of denaturation at 94°C, 45 s of annealing at 59°C, 40 s of extension at 72°C, and 10 min of final extension at 72°C.

The products of PCR amplification were separated on 2% agarose gel and were visualized in ultraviolet light after ethidium bromide staining.

C Statistical Analysis

Statistical analyses were performed using descriptive statistic and analyses of variance (ANOVA) to characterize the sample and to evaluate the relationships of the psychometric scales scores with VNTR MAO-A genotypic variants as a

function of follow-up time, using the SPSS 13.0 package (SPSS, Inc., Chicago, IL, USA). Statistical significance was set at the $p < 0.05$ level.

III. RESULTS

The allelic variants of VNTR MAO-A polymorphisms of the subjects considered are shown in Table 1; the genotypic distribution for VNTR did not significantly differ from the Hardy–Weinberg equilibrium ($\chi^2 = 2.65$; $p = 0.18$).

The 53 patients considered were initially subjected to psychometrical evaluation at recruitment (T0), and 35 of them (66%) were retested psychometrically at follow-up 3 months later (T1). When the scores of subscale employed determined at recruitment (T0) were stratified for age, employment, marital status, education, disease stage, treatment, and genotype, no significant differences were revealed (Schillani et al., 2012).

When the effects of time from enrollment to follow-up were considered, the analysis of the relationships between VNTR MAO-A polymorphism and the Mini-MAC scale indicated that the scores of anxious preoccupation of Mini-MAC decreased significantly with time and significantly depended on the genotype of the patients. Women with the genotype conferring low functional activity to MAO-A (homozygous 3–3 repetitions, and heterozygous 3–4 and 3–3.5 repetitions) displayed a significantly larger decrease of anxious preoccupation at follow-up (ANOVA: $F = 5.527$; $df = 2$; $p = 0.06$) compared to the carriers of the highly functional variant (homozygous 4–4 repetitions) (ANOVA: $F = 1.935$; $df = 2$; $p = 0.154$) (Table 2). No statistically significant effects of VNTR genotype and time observed for the other subscales of Mini-MAC.

IV. DISCUSSION

The activity of monoamine oxidases appears to be relevant in a constellation of phenomena, such as monoaminergic neurotransmission in the brain (Finberg

TABLE 1: Frequency of VNTR MAO-A Polymorphism

Genotype	Allelic variants	Functionality*	N	%
Homozygous	3–3 repetitions	Low	9	17
Heterozygous	3–4 repetitions	Low	21	39.7
Heterozygous	3–3.5 repetitions	Low	1	1.8
Homozygous	4–4 repetitions	High	22	41.5

*Sabol (1998)

TABLE 2: Anxious preoccupation scores of MINI-MAC in relation to time and MAO-A genotype (mean $\pm$ SE)

	T0$^{\#}$	**T1**$^{\#}$	**P**^{a}	**P**^{b}
All genotypes	15.45 $\pm$ 0.66 (N = 53)	11.93 $\pm$ 0.64 (N = 40)	**.006**	
Low functionality$^{\wedge}$	15.19 $\pm$ 0.97 (N = 31)	10.96 $\pm$ 0.68 (N = 26)	**.006**	**.050**
High functionality$^{\wedge}$	15.82 $\pm$ 0.86 (N = 22)	13.71 $\pm$ 1.22 (N = 14)	.154	

$^{\#}T0$ = enrollment into the study $T1$ = 3 months later. The data were analyzed using ANOVA, testing the effect of time
^{a}(F = 5.284; df = 2) and genotype
^{b}(F = 1.109; df = 1) as independent variables; statistical significance was set at $p <$ 0.05.
$^{\wedge}$See Table 1, and Sabol (1998), for definition.

et al., 1998), depressive conditions, and response to antidepressant MAO inhibitors (Balciuniene et al., 2001; Yamada and Yasuhara, 2004). The VNTR genetic polymorphism influenced monoamine oxidase activity (Caspi et al., 2002; Lesch et al., 1996) and conferred vulnerability to antisocial problems in maltreated children in a gene via environmental interaction (Lesch et al., 1996).

Women with early breast cancer experience the stress of the cancer diagnosis and of the initiation of treatment. Their mental adaptation to the disease is characterized by anxious preoccupation, which spontaneously decreases with time. This reduction of anxious preoccupation has been determined to be influenced by the genetic polymorphism 5-HTTLPR of serotonin transporter (Schillani et al., 2012).

The results obtained in the present study also show that monoamine oxidase activity is significantly involved in the spontaneous reduction of anxious preoccupation in women with early breast cancer. The analysis of the data does not display significant interactions between time and MAO-A genotype, which might depend on the relatively small sample size; For anxious preoccupation, no significant correlation was observed between low-functionality MAO-A VNTR and 5-HTTLPR carriers.

Thus, the genotyping of women with early breast cancer for 5-HTTLPR and MAO-A VNTR may allow the identification of vulnerable subjects, which display a less pronounced reduction of their initial anxious preoccupation and higher scores at follow-up. On this basis, interventions may thus be aimed at the subjects in greater need of support. In the case of drug therapy, these results may allow the choice of the drug with the most appropriate mechanism of action and pharmacogenetic properties.

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