Influence of catechol-o-methyltransferase genotype (Val158Met) on endocrine, sympathetic nervous and mucosal immune systems in breast cancer survivors

César Fernández-de-las-Peñas, Irene Cantarero-Villanueva, Carolina Fernández-Lao, Silvia Ambite-Quesada, Lourdes Díaz-Rodríguez, Inés Rivas-Martínez, Rosario del Moral-Avilá, Manuel Arroyo-Morales

**Abstract**

Stress can play an important role in development of cancer-related fatigue (CRF) by activating the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system (SNS), and altering the immune system. This study examined the influence of catechol-O-methyltransferase (COMT) Val158Met genotypes on salivary markers of HPA axis (cortisol), SNS (α-amylase) and immune (IgA) systems, as well as on CRF in breast cancer survivors (BCS). One-hundred BCS participated. After amplifying Val158Met COMT polymorphisms by polymerase chain reaction, three COMT genotypes were considered: Val/Val, Val/Met, Met/Met. Salivary cortisol, α-amylase activity, salivary flow rate, and IgA concentration were collected from non-stimulated saliva. CRF was assessed with the fatigue subscale of the Profile of Mood State (POMS) questionnaire. We found that BCS carrying Met/Met genotype reported higher cortisol concentration, α-amylase activity and greater CRF than those with Val/Met \( (P < 0.05) \) and Val/Val \( (P < 0.001) \) genotypes. No differences in salivary flow rate or IgA concentration \( (P > 0.20) \) were found. The results suggest that BCS carrying Met/Met genotype exhibit greater dysfunction of the HPA axis and SNS system associated with severe CRF. This study is important because it strives to understand biological factors that predispose some BCS to higher levels of CRF.

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**Introduction**

Despite an increasing body of research investigating cancer-related symptoms in breast cancer survivors (BCS), particularly cancer-related fatigue (CRF), there remain deficits in the literature. Cytokine deregulation, 5-HT neurotransmitter deregulation, circadian rhythm disruption, alterations in adenosine triphosphate (ATP) metabolism, vagal afferent activation and hypothalamic-pituitary-adrenal (HPA) axis dysfunction are proposed mechanisms for explaining CRF.

It has been postulated that stress can play an important role in the development of cancer recurrence, as it activates HPA axis and sympathetic nervous system (SNS). Cortisol and α-amylase have been suggested as salivary biomarkers of HPA axis and SNS activity. In fact, reduction and altered circadian response of cortisol levels, which is associated with HPA axis dysfunction has been identified in BCS with CRF. In addition, a recent study reported that HPA axis (cortisol) and SNS deregulation (plasma epinephrine and nor-epinephrine) were related to greater CRF and depression in BCS. Additionally, studies have found a decrease in immunoglobulin A (IgA) after treatment of breast cancer, suggesting the presence of immunological abnormalities in BCS.

Barsevick et al recently suggested the need for further research on biological and genetic mechanisms of CRF to further elucidate the relationship between biological markers and emerging understanding of genetic expression in CRF. In this direction, the role of the catechol-O-methyltransferase (COMT) gene in cancer remains unclear. The Val158Met polymorphism leads to a substitution of valine (Val) with methionine (Met) at codon 158 on chromosome 22q11. This enzyme is involved in the metabolic degradation of
It is known that genetic polymorphism due to a C → A substitution at codon 158 of the COMT gene, leading to a Val to Met substitution, results in lower enzymatic activity than the Met allele (Val/Val, Met/Met) results in a high-activity COMT variant related to a 3–4 times higher enzymatic activity than the Met allele (Val/Met, Met/Met) which results in low-activity variants. Because of the effects of the COMT gene on estrogen metabolism, it has been hypothesized that women with lower activity of this enzyme (Met/Met) may be at a higher risk of breast cancer; however, two recent meta-analysis have concluded that COMT Val158Met polymorphism is not associated with breast cancer risk.

Although no differences in Val158Met genotype distribution exist between women with and without breast cancer however, there is evidence showing the genetic influence of the COMT enzyme in cognitive function in BCS17 supporting the relevance of this gene in the clinical expression of breast cancer. As the presence of psychological stress is associated with greater CRF,18 studies investigating genetic contributions of the COMT Val158Met polymorphism on cancer-related symptoms are necessary.

In the current study, we examined the influence of COMT genotype on salivary markers of HPA axis (cortisol), SNS (α-amylase) and immune (IgA) systems as well as on CRF in BCS. Our hypotheses were: 1. BCS with Met/Met genotype exhibit higher salivary cortisol concentrations, and α-amylase activity associated with a greater CRF than those with Val/Val or Val/Met genotypes; and, 2. BCS carrying Met/Met genotype would exhibit reduced salivary flow rate and lower IgA concentrations associated with more severe CRF than those with Val/Val or Val/Met genotypes.

**Material and methods**

**Participants**

Patients were recruited from the Unit of Breast Oncology of the Hospital Virgen de las Nieves, Granada, Spain. They were eligible if they: 1, had a diagnosis of breast cancer; 2, had undergone a simple mastectomy or quadrantectomy with reconstruction; 3, between 25 and 65 years old; 4, finished co-adjuvant treatment at least one month before the study except endocrine therapy; and, 5, did not have active cancer. They were excluded if they: 1, were receiving chemotherapy or radio- therapy treatment at the time of the study; 2, breast surgery for cosmetic reasons or prophylactic mastectomy; 3, any medical inflammatory condition (arthritis); 4, recurrent cancer; or 5, previous diagnosis of fibromyalgia syndrome.

The study protocol was approved by the local Ethics Committee (Hospital Virgen de las Nieves, Spain) and conducted following the Helsinki Declaration. All participants signed an informed consent prior to their participation.

**Saliva collection**

Non-stimulated saliva samples were collected into collection tubes (by passive drooling technique) for 3 min and used for assessment of HPA axis, SNS and immune functions according to standardized procedures.20 All saliva sampling was performed between 10 and 12 am and always 4 h after wake-up to control possible fluctuation associated to diurnal rhythms on cortisol and α-amylase secretions.5 It has been identified that 4 h after waking up α-amylase secretion reaches highest levels of the day.5 Patients were asked not to eat, drink or chew gum for 1 h before sampling. Additionally, they were asked to refrain from brushing their teeth the morning before saliva sampling. Saliva samples were centrifuged at 3000 rpm for 15 min to remove sediments and then were stored at −70°C until analysis (supernatant). Concentrations of cortisol and IgA and α-amylase activity were assessed in the thawed samples.

Salivary cortisol and IgA concentrations, and α-amylase activity were calculated using a commercial luminescence immune assay (Salimetrics, State College, PA, USA), reading the luminescence units with automatic luminometers (Sunrise, TECAN Group, Man- nedorf, Switzerland). Saliva samples were analyzed in a single batch to eliminate inter-assay variance and they were measured in duplicate. In fact, adequate intra-assay accuracy was obtained with a coefficient of variance between 5.5 and 7%.

**DNA collection and COMT genotyping**

Genomic DNA was extracted from saliva cell sediments using "Genomic DNA extraction and purification Kit" (Real Molecular Biology) following the manufacturer's instructions. The single Val158Met (rs4680) nucleotide polymorphism was genotyped using a TaqMan™ Drug Metabolism Genotyping Assays on a Real Time PCR ABI Prism 7000 Sequence Detection System (Applied Biosystem, USA) in the Genomic Center, Centro de Apoyo Tecnológico. The 3 possible halotypes were associated with different fluorescent dyes to identification of genotypes: Val/Val, Val/Met, or Met/Met.

**Cancer-related fatigue**

Fatigue was assessed with the fatigue subscale of Profile of Mood State (POMS) questionnaire. The POMS questionnaire (Spanish version software) consists of 63 items on mood state. Scores (on a five-point scale ranging from 0 to 4) are grouped into 6 subscales and converted into T-scores for the analysis. Reliability of the Spanish version of the POMS has been found to be high (Cronbach’s α 0.76–0.91).21

**Statistical analysis**

Data were analyzed with the SPSS statistical package (17.0 Version). Results are expressed as mean, standard deviation and 95% confidence interval. The Kolmogorov–Smirnov test showed that quantitative variables had a normal distribution of the data (P > 0.05). A 1-way ANOVA was used to assess differences in age, educational level and cancer features by Val158Met genotype. Variables significantly different among groups were entered as covariates in later analyses. A one-way (genotype) ANOVA was used to examine differences in salivary flow rate, cortisol concentration, α-amylase activity, IgA concentration and cancer-related fatigue among the 3 groups. Post-hoc comparisons were conducted with the Holm-Bonferroni correction.22 Finally, the Pearson (r) correlation test was used for investigating the relationships between fatigue, cortisol, IgA and α-amylase activity depending on the Val158Met genotype. The analysis was conducted at a 95% confidence level. A P < 0.05 was considered statistically significant.

**Results**

One hundred (n = 100) BCS, aged 30–63 years (mean age: 48, SD: 8 years) were included in the study. Forty-seven patients underwent sentinel lymph node biopsy, whereas the remaining 53 underwent axillary lymph node dissection. All patients had received both radiotherapy and chemotherapy as co-adjuvant treatment after the surgical intervention. Patients had finished their co-adjuvant treatment a mean of 2 months (SD: 0.4 months) before their inclusion in the study. Thirty-two (32%) had Val/Val genotype, 46 (46%) had a Val/Met genotype, and the remaining 22 (22%) had Met/Met genotype. Demographic features of the three groups are presented in Table 1. The ANOVA revealed that groups
were not significantly different in terms of age, time from the diagnosis, time from surgery, type of surgery, stage of cancer, post-menopausal status or current endocrine therapy.

Means, standard deviations, and 95% confidence intervals revealed that their statistical comparison for salivary flow rate, cortisol concentration, α-amylase activity and IgA concentration are summarized in Table 2. A significant effect of COMT genotype was found for cortisol concentration and α-amylase activity (P < 0.01), but not for salivary flow rate or IgA concentration (P > 0.20); BCS carrying the Met/Met genotype exhibited significantly higher cortisol concentration and α-amylase activity than those BCS with Val/Met (P = 0.003, P = 0.038, respectively) and Val/Val (both, P < 0.001) genotypes.

The ANOVA test reported a significant effect of genotype for fatigue domain of the POMS (F = 8.907; P < 0.001). Post hoc comparisons with Holm-Bonferroni correction revealed that BCS with Met/Met genotype showed significant higher fatigue scores (mean: 57.8 ± 7.1) than those with Val/Met (mean: 52.9 ± 8.7, P < 0.05) and with Val/Val (mean: 48.3 ± 7.7, P < 0.001) genotypes.

A significant positive association between the fatigue domain of the POMS and cortisol concentration (r = 0.416; P = 0.004) was found in BCS with Val/Met, but not Val/Val (r = 0.221; P = 0.209) or Met/Met (r = 0.393; P = 0.186), genotype: the greater the fatigue, the higher the cortisol concentration. No other relationship between fatigue and salivary biomarkers was found.

### Discussion

The results of this study indicate that BCS carrying Met/Met genotype exhibit higher cortisol concentration, α-amylase activity and CRF than those with Val/Val or Val/Met genotype. No differences in salivary flow rate and IgA concentrations were found. These results provide initial evidence of a link between COMT genotype, HPA axis and SNS dysfunction and CRF in BCS.

The results indicating that BCS carrying Met/Met genotype exhibited higher cortisol concentration and α-amylase activity as well as greater CRF further support the multidimensional aspect of fatigue. The existence of imbalance within the HPA axis in BCS has already been reported as higher cortisol concentrations have been previously found in BCS compared with controls.67 Our findings extend our current knowledge by demonstrating that the imbalance in HPA axis is greater in BCS carrying Met/Met genotype. A higher imbalance within the HPA axis may be related to worse adaptation responses to stress in this population of BCS.

The current study is the first one investigating α-amylase activity as biomarker of SNS dysfunction in BCS. Again, higher α-amylase activity was found in BCS with Met/Met genotype. A mechanism by which Val150Met genotype could be related to SNS dysfunction may be that reduced COMT gene activity would result in elevated levels of catecholamines, such as epinephrine or nor-epinephrine promoting the production of pain via stimulation of β2-adrenergic receptors in the peripheral and central nervous systems.23 In fact, Thornton et al. have shown the presence of higher levels of plasma epinephrine and nor-epinephrine in BCS; whereas Nackley et al reported that low COMT activity increased pain sensitivity by activating β2 and β3-adrenergic receptors, supporting this hypothesis.22 However, the interactions of the SNS are highly complex, as control of epinephrine, nor-epinephrine or α-amylase activity is determined by several enzymes.

We also found that BCS carrying Met/Met genotype also exhibited greater levels of CRF. In fact, differences in CRF depending on Val158Met genotype (range 5–10 points) reached the minimally important clinical difference (MICD, mean: 5.6 points) for the overall scale of the POMS.25 On several note explaining CRF is an increase in cytokine concentration which can cause hyper-excitability in nociceptive transmission and exaggerated release of substance P and excitatory aminoacids.26 It seems that reduction in Val158Met gene activity (Met/Met genotype) leads to a reduction in the content of endogenous opioids-like peptides in some regions of the central nervous system.27 Therefore, it is possible that biological changes presented by BCS carrying the Met/Met genotype, exaggerated pain responses, and higher HPA axis and SNS dysfunction, influence CRF. The results of the current study further support the influence of HPA axis and SNS disruption on CRF and provide initial evidence of a genetic influence in the relationship between these factors. Nevertheless, it seems that a single nucleotide polymorphism cannot account for CRF alone: so, it is possible that multiple polymorphisms impact HPA axis, SNS and CRF.

Finally, we did not find differences in salivary flow rate and IgA concentrations depending on Val158Met genotype. A previous study found a decrease in salivary flow rate and IgA concentration in response to chemotherapy.10 It has been suggested that adjuvant chemotherapy depresses IgA producing plasma cells located within salivary gland tissues or inhibits immunoglobulin transport mechanisms in the salivary gland cells.28 However, these drug reactions were temporary as salivary findings returned to baseline values one-year following treatment.10

The results of our study can be placed in the context of individualized medicine29 where genetic research permits to identify populations of patients who can benefit from symptom intervention by developing personalized approaches for patients with severe symptoms.30 The results of this study suggest that BCS carrying Met/Met genotype is more likely to suffer more HPA axis and SNS imbalances and greater CRF. This is a clinical and important finding as carriers of Met/Met genotype by BCS may be associated with better cognitive function31 but greater biological imbalances and higher CRF.

Although the results of the present study are promising, a number of limitations should be recognized. First, the sample was mainly composed of white BCS; therefore, extrapolation of current results to more diverse populations should be done cautiously. Nevertheless, recent reviews have reported no ethnicity differences in genetic models suggesting that genotype distribution is consistent between Asians and Caucasians.31,32 We did not conduct a power sample calculation since no previous data were available. The current study can help future studies to calculate their

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Val/Val (n = 32)</th>
<th>Val/Met (n = 46)</th>
<th>Met/Met (n = 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean (SD)</td>
<td>47 (9)</td>
<td>49 (8)</td>
<td>48 (10)</td>
<td>0.646*</td>
</tr>
<tr>
<td>Time [m] from diagnosis</td>
<td>12 (2)</td>
<td>13 (6)</td>
<td>12 (3)</td>
<td>0.932*</td>
</tr>
<tr>
<td>Time [m] post-surgery</td>
<td>8 (4)</td>
<td>8 (5)</td>
<td>8 (3)</td>
<td>0.962*</td>
</tr>
<tr>
<td>Type of surgery, n (%)</td>
<td>22 (69)</td>
<td>29 (63)</td>
<td>14 (63)</td>
<td>0.789*</td>
</tr>
<tr>
<td>Tumorectomy</td>
<td>10 (31)</td>
<td>17 (37)</td>
<td>8 (37)</td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>18 (56)</td>
<td>23 (50)</td>
<td>12 (54)</td>
<td>0.557*</td>
</tr>
<tr>
<td>Side of surgery, n (%)</td>
<td>14 (44)</td>
<td>23 (50)</td>
<td>10 (46)</td>
<td></td>
</tr>
<tr>
<td>Tumor stage, n (%)</td>
<td>15 (47)</td>
<td>22 (48)</td>
<td>10 (45)</td>
<td>0.841*</td>
</tr>
<tr>
<td>I</td>
<td>13 (40)</td>
<td>18 (40)</td>
<td>9 (42)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4 (13)</td>
<td>6 (12)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Post-menopausal status, n (%)</td>
<td>17 (70)</td>
<td>34 (71)</td>
<td>10 (67)</td>
<td>0.702*</td>
</tr>
<tr>
<td>No</td>
<td>15 (30)</td>
<td>12 (29)</td>
<td>12 (33)</td>
<td></td>
</tr>
<tr>
<td>Current endocrine therapy, n (%)</td>
<td>16 (53)</td>
<td>25 (54)</td>
<td>11 (50)</td>
<td>0.803*</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>14 (47)</td>
<td>21 (46)</td>
<td>11 (50)</td>
<td></td>
</tr>
</tbody>
</table>

*P values for comparisons among group based on chi-square* and analysis of variance* tests.
appropriate sample sizes for detecting differences in specific outcomes which did not reach statistical significance. Second, all patients received chemotherapy and radiotherapy as part of their treatment for breast cancer. We do not know if BCS who had received only 1 adjuvant therapy will present similar results. In addition, patients had finished their co-adjuvant therapy 2 months before the study which can be considered relatively soon. It would be important to determine if the observed associations in the current study remain stable with time as some of them may be transient due to some BCS were recovering from treatment side-effects. Third, we did not include a healthy group as we focused on BCS. Finally, we only investigated the Val158Met (rs4680) nucleotide polymorphism. Future studies should investigate the influence of different Val158Met polymorphisms in addition to other genetic factors in biological markers and CRF in BCS.

Conclusions

This study identified that BCS carrying the Met/Met genotype exhibit higher cortisol concentration and α-amylase activity as well as CRF compared with those with Val/Val or Val/Met genotype. No significant differences in salivary flow rate and IgA concentrations were found. The current results provide initial evidence of the influence of Val158Met polymorphism, HPA axis and SNS dysfunction and CRF in BCS.

Conflict of interest statement

None declared.

Contributors

CFdlP, ICV, CFL, SAQ, LDR, IRM, RMA, and MAM contributed to conception and design. CFdlP, ICV, CFL and MAM wrote the protocol. RMA, CSS and ICV were responsible for the recruitment of the patients. CFdlP, CSS, ICV, SAQ, IRM and LDR collected the data. CFdlP and MAM provided clinical expertise. MAM was an expert statistician. CFdlP, ICV, CFL, LDR, and MAM contributed to the statistical analysis and interpretation of data. CFdlP, ICV, CFL, SAQ, LDR, IRM, RMA, and MAM drafted; revised, and supplemented the manuscript. All authors approved the final version.

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References


