Reviewer’s report

Title: Leptin and Leptin Receptor Polymorphisms Associated with Increased Risk and Poor Prognosis of Breast Carcinoma

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Reviewer: rui medeiros

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“Leptin and leptin receptor polymorphisms associated with increased risk and poor prognosis of breast carcinoma”

COMMENTS:

Leptin involvement in breast cancer has been widely suggested, although only a multistest genetic study in LEP and LEPR and an association study in LEPR evaluated the relevance of these low-penetration genes in breast cancer.

The manuscript attempts to ascertain susceptibility and prognostic value for sporadic breast cancer in carriers of genetic variants in LEP and LEPR genes. The polymorphisms studied imply a functional modification in protein expression or in intracellular signalling.

The association of LEP and LEPR polymorphisms to breast cancer is not completely original. There is a recent paper addressing the LEPR 223 polymorphism in breast cancer patients, curiously with results opposing to these submitted, and other evaluating polymorphisms different from the submitted in LEP and LEPR genes. However, the present manuscript uses a considerably greater sample (n=308 patients, n=222 controls), conferring it potential. Nevertheless, the submitted study was undertaken in a different ethnic group.

In general the authors made a small effort to combine results from the study with previous published reports on breast cancer patients of LEPR polymorphisms, serum leptin levels or in vitro demonstration of intracellular signalling pathways involved in breast cancer, which are conflictuous.

The information from other studies is not always well discussed and no uniform etiopathological mechanism/theory is proposed concerning the contribution of this and other studies. The authors should attempt to elaborate a clear hypothesis based upon the integration of the present results and previous evidences.

The language should be thoroughly edited. There are several words and phrases which don’t sound right. The difficulties in spelling and grammar make the ideas not fluent and the home take message tricky.

(p.e. in title, “…polymorphisms are associated…”; in the abstract the conclusion section, “…breast cancer risk as well as…”; in page 4 line 5 re-write the phrase; in results section page 11 line 4 “…Hardy-Weinberg equilibrium in both patient …”; in page 12 line 5, the word relationship is separated by an extra space; in page 14 line 3, hiperleptinimia should be written hiperleptinemia; in page 14 line 14 re-write the phrase; in page 15 line 5 “…associated with a larger tumor size…”; in page 15 line 8 re-write the phrase; in page 15 line 18 re-write the phrase without comas and introduce which is after “…cell proliferation, which is …”; page 16 line 4 “…Factors such as …”; in page 17 line 2 remove the words “its receptor” remaining “…LEP an LEPR…”; the first sentence of the last paragraph in the discussion section is very large.

The assumption of the functional characteristics in LEP -2548 G/A polymorphism is consistent with results, and it is an original contribution. However, in the LEPR (Q223R) polymorphism, in which a glutamine to arginine substitution occurs, the functional capability is not thoroughly established, although an impaired signalling capacity has been suggested. Furthermore, the authors found an association of LEPR 223R allele with breast cancer susceptibility and prognosis, while no biological
plausibility seems to support these findings. The controversy arises from the hypothesis that the LEPR 223R allele carriers have higher risk for breast cancer and poor prognosis, while this allele is related to impair signalling capacity. If this allele has lower activity, how can we explain the proliferative and pro-angiogenic capacity of leptin. It was expected a protective effect for this allele. The authors should clarify this issue.

There are all over the background and discussion sections references to studies of leptin involvement in other cancer types. The authors could have used almost references to in vitro and in vivo studies undertaken in breast cancer, since there is a great body of evidence in this oncologic model.

The procedures used in statistical analysis are adequate, however, the authors should present the P-value of Hardy-Weinberg testing, and the results could be adjusted to other confounding variables (p.e. age at menarche, reproductive history, oral contraceptives, hormonal substitution therapy). It would be interesting to study the combination of the alleles from both polymorphisms - study the haplotype LEPR/LEP. The authors used TNM clinicopathological staging, however it would be suitable to use the more uniformly accepted staging I – IV? Is the T1/T2 vs. T3/T4 stratification the one with the most clinical chirurgical and prognostic significance?

The authors should discuss the negative results found after adjustment for menopause status and clinicopathological data. The authors should explain the reason why there is no risk after stratification for menopause status; how was performed the stratification? It is not described in statistical analyses section.

In page 15 there it is reported an evaluation of the predictive value of LEP and LEPR polymorphisms in clinical response to chemotherapy. However, there is no description of material or methods used, or a statement of the drugs used and posologic regimen. We believe discussion should be increased comparing results from this paper with other previously reported results in other hormonal neoplasia regarding leptin gene polymorphism.

**What next?:** Accept after minor essential revisions

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No