Author's response to reviews

Title: Serum 25-hydroxyvitamin D3 levels and Vitamin D Receptor variants in melanoma patients from the mediterranean area of Barcelona

Authors:

Zighereda Ogbah (zigheog@gmail.com)
Laura Visa (lvisa@clinic.ub.es)
Celia Badenas (cbadenas@clinic.ub.es)
José Ríos (jrios@uab.es)
Joan-Anton Puig-Butille (jantonpuig@gmail.com)
Nuria Bonífaci (nbonifaci@iconcologia.net)
Elisabet Guinò (eguin@iconcologia.net)
Josep Maria Augè (jmauge@clinic.ub.es)
Isabel Kolm (ikolm@yahoo.com)
Cristina Carrera (ccarrera@clinic.ub.es)
Miquel Angel Pujana (mapujana@iconcologia.net)
Josep Malvehy (jmalvehy@clinic.ub.es)
Susana Puig (spuig@clinic.ub.es)

Version: 4 Date: 18 December 2012

Author's response to reviews: see over
Barcelona, 18 December 2012

Dear BMC Medical Genetics Editor and Reviewers,

Thank you for taking the time to review our manuscript “Serum 25-hydroxyvitamin D3 levels and Vitamin D Receptor variants in melanoma patients from the Mediterranean area of Barcelona”. We are grateful to reviewers for their helpful comments and suggestions. We have taken all of these into account when revising the manuscript. We have addressed the specific points raised by each reviewer below. All our replies to the comments of the reviewers and all changes in the revised manuscript have been written in red.

Kind regards,

Zighereda Ogbah

Reviewer’s report 1

Manuscript title:
**Serum 25-hydroxyvitamin D3 levels and Vitamin D Receptor variants in melanoma patients from the Mediterranean area of Barcelona**

This manuscript presents an analysis of two studies on melanoma patients. The aims were: to investigate Vitamin D levels and the relationship between VDR variants and number of naevi, the strongest phenotypic risk factor for melanoma.

There are some concerns about analyses of the data and conclusions driven.

Major Compulsory Revisions

1. The authors concluded that their study showed that even in Barcelona, a sunny Mediterranean area, Vitamin D levels were sub-optimal in the majority of melanoma patients at diagnosis. However the choice on the cut-off point should be discussed and analysed more deeply. The authors choose 25ng/ml to define low vitamin D serum level but many expert indicated 30 ng/ml as cut off point, based on reviews and meta-analyses on cancer incidence and overall mortality. On the other hand the IOM, after reviewing the published literature, concluded that the serum level of vitamin D sufficient for bone health is above 20 ng/ml. A rational on the choice of cut-off points and a discussion on those aspects should be considered in the paper.

We agree with the reviewer’s comment. In our study a level of ≤25 ng/ml as 25-hydroxyvitamin D3 insufficiency was chosen as reported in a previous study in melanoma patients, but we have added a comment about discrepancies when selecting the cut off level in the discussion (page12 line 251-261)

“Several meta-analyses support the evidence that low 25-hydroxyvitamin D3 serum level, which is highly prevalent worldwide, is a risk factor for many chronic diseases including cancer [35]. The minimum desirable serum level of 25-hydroxyvitamin D3 has been suggested to be between 20 ng/ml and 30 ng/ml [36]. In 2011 The IOM committee reported guidelines stating that a level of 25-hydroxyvitamin D3 >20 ng/ml is needed for good bone and general health for practically all individuals [37]. However, the IOM report did not consider clinical and demographic variables (ie: race/ethnicity, adiposity, body composition, sun exposure, etc) by which 25-hydroxyvitamin D3 levels are notoriously affected. Recently, a meta-analysis involving 59 231 healthy subjects from eleven prospective cohort studies suggested that optimal 25-hydroxyvitamin D3 concentrations of between 30 ng/ml and 40 ng/ml (75 nmol/L and 100 nmol/L) are needed to reduce mortality [38].”

2. Statistical analyses:

- Mean values and SD are presented for continuous variables but means are representative of the population when variables have a normal distribution, otherwise
median and range interquartile should be considered. Furthermore normal distribution of residuals should be checked for multivariate approach for continuous response variables.

Medians and range interquartile range have been reported in table 1, table 2 and table 4 as well as in the manuscript (Results paragraph).

- Why the authors did not consider the analysis evaluating the association between 25(OH)D and VDR with Breslow thickness (the main prognostic factor), considering Breslow as response variable (as continuous variables and categorizes)? And what about considering number of melanoma (in a logistic model) as response variable?

Vitamin D levels depend on many independent factors (age, BMI, season, pigment features, sun exposure, sunscreen use). As reported, to see the effect of vitamin D levels we analyzed both, Breslow thickness and number of melanomas.

Vitamin D levels were not different in relation to number of melanomas or Breslow (continuous variable). Association between Breslow as categorical variable and Vitamin D levels has been now included (Methods page line; Results page from line; Discussion page from line; tables 1 and 2). Again, no association between Breslow and Vitamin D was found. There is no data on Vitamin D levels and Breslow in melanoma patients from Spain. Although our result is interesting, we do not exclude that the lack of relationship between Vitamin D level and Breslow thickness can result from the small cohort of melanoma patients. Breslow thickness variable was considered only in the first study or Vitamin D study.
Reviewer’s report 2

Title: Serum 25-hydroxyvitamin D3 levels and Vitamin D Receptor variants in melanoma patients from the Mediterranean area of Barcelona

Version: 3 Date: 27 September 2012
Reviewer: Anna Brozyna

Reviewer’s report:
This is an interesting, extensive, well-designed and executed study that most of melanoma patients from sunny Mediterranean area had insufficient vitamin D3 level and that some VDR SNPs can protect against multiple melanomas. The study was performed using appropriate techniques in a sufficient number of tissue samples in SNPs analysis, with smaller group in vitamin D study. The discussion and conclusions are balanced and reasonable. Overall, this is a well-written paper reporting results of a comprehensive, carefully done experimental work. Vitamin D level depend on many independent factors (age, season, diet, sunscreen use and others), thus the lack of relationship between vitamin D level and Breslow thickness can result from small cohort of melanoma patients.

I have additional recommendations for revision of the paper:

Major Compulsory Revisions
1. Page 10. Melanomas at stage IIIA is not localized disease. At this stage patient has lymph node metastases. Thus it should be clarified.

The reviewer is right. This sentence has been added in Methods (page 5 lines 71-73) “All patients had localized tumors (stage I and stage II) except one (stage IIIA) which had lymph node metastasis.”

2. Summarize patients characteristic in table – it would be more clear.

As suggested, we have summarized patients characteristic in tables.

3. Add a table summarizing clinic and pathologic data for melanomas (stage, Breslow, site).

A total of 5 tables have been included in the paper. In order to make them clearer, we have modified the existing tables describing first clinical characteristic, habits of solar exposure and photoprotection and then melanoma characteristic.

4. Some studies showed that vitamin D level affect survival in cancer patients (including melanoma patients) thus I think this paper’s impact would be increased by examining the disease free survival and overall survival in relation to vitamin D3 level and SNPs (at least for significant SNPs).

Unfortunately, data on melanoma survival are not documented in our database as all patients are still alive except one who died from a stroke.

Minor Essential Revisions

1. List the analyzed SNPs in method section.
SNPs have been listed in methods section (page 6 from lines 98-103).

2. Fig. 1 legend – explain meaning of circles and asterisk.
The circles represent high Vitamin D values (39.9ng/ml and 41ng/ml) and the asterisk the highest Vitamin D value 61.7 ng/ml. We have added these data in Fig 1 legend (page 20, lines 540-541).

3. Some grammar and syntax corrections are needed, e.g.:
Page 2: “comparing melanoma patients 150 with low and 113 with high nevus number”
This sentence has been modified in the new version of the abstract.

4. Page 7: “We selected 11 SNPs based on linkage disequilibrium (LD) with 0.5 > r² > 1 for Caucasians using Hapmap (http://www.hapmap.org) and the Tagger program. We included SNPs that had at least one independent validation criterion as established in dbSNP (htpp://www.ncbi.nlm.nih.gov/snp) and reported minor allele frequencies (MAF) ≥ 0.05”

The sentence has been corrected as following (Methods, page 6 lines 98-103):
“In total, 11 VDR SNPs rs7136534, rs11574027, 11168287, rs2238136, rs3782905, rs2189480, rs2239179, rs11574077, rs11168267 and rs7975128 were analyzed. All SNPs were selected as having minor allele frequencies (MAF) ≥ 0.05 in HapMap (www.hapmap.org) European individuals and at least one independent validation criterion, as established in dbSNP (htpp://www.ncbi.nlm.nih.gov/snp). The SNPs were selected using data from the HapMap project and the Tagger tool [32] with linkage disequilibrium r² > 0.5.”

Discretionary Revisions
1. Nevus size is one of risk factor for melanoma development. In his paper there is lack of information regarding nevi size in studied cohorts. Please clarify what was the nevi size (minimal, maximal, mean). There were any differences in group with low and high nevi number?

Nevi were examined by dermatologists and trained nurses. According to the total nevus number, in clinical practice of our department patients nevus number are classified into categories (<50, 50-100, >100). For this study, we selected two groups of patients where there would be no possible overlap between them, as <50 nevi were closer to the normal nevus number in our population [Aguilera P. et al, 2009] and >100 nevi being a well-documented risk factor for melanoma [Gandini S et al., 2005]. Differences between patients with low and patients with high nevus number were found for age of melanoma diagnosis and number of melanoma (Results, page 11 from line 222). Unfortunately, information about nevus size is not included in the phenotypic characterization of the patients when they were included in the database.

2. Please clarify when nevi were counted, at the time of melanoma diagnosis or after?
Nevi were counted for all patients at time of melanoma diagnosis. Now this information has been included (Methods, page 5, line 57 and page 6, line 84).

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.
Reviewer's report 3
Title: Serum 25-hydroxyvitamin D3 levels and Vitamin D Receptor variants in melanoma patients from the mediterranean area of Barcelona
Version: 3 Date: 4 October 2012
Reviewer: Aleksandra Batycka-Baran
Reviewer's report:
It is the nice and interesting study. However, you should rebuild abstract and the english should be improved. In the main text there is a lot of language - grammar mistakes - please send it to experienced english medical writer. For me P= 0.049 shows a doubtful statistical significance. SNP is single-nucleotide polymorphism not just polymorphism - please correct it. The part concerning vitamin D metabolism could be more precise. In my opinion instead of vitamin D that is general term the authors should use the term 25-Hydroxyvitamin D3.

SNP as single-nucleotide polymorphism has been corrected and Vitamin D has been replaced with 25-Hydroxyvitamin D3. We agree reviewer that P-values around 0.049 are of doubtful statistical significance. Skin type (P=0.052, Table 2) is a well-known melanoma risk factor. To this, skin type variable was included in multivariable and, as expected, it results not to influence Vitamin D levels. The paper now has been sent to English writer for language corrections. The part concerning Vitamin D metabolism has been revised (Introduction, page 3 from line 14).