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P-selectin genotype is associated with the development of cancer cachexia

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 12 January 2012

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript.

As you will see from the enclosed reports, overall the referees are in favor of publication, therefore, we feel that we can consider a revision of your manuscript if you can address the issues that have been raised within the space and time constraints outlined below. Please note that it is EMBO Molecular Medicine policy to allow only a single round of revision and that, as acceptance or rejection of the manuscript may depend on another round of review, your responses should be as complete as possible.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions, except under exceptional circumstances in which a short extension is obtained from the editor. Also, the length of the revised manuscript may not exceed 60,000 characters (including spaces) and, including figures, the paper must ultimately fit onto optimally ten pages of the journal. You may consider including any peripheral data (but not methods in their entirety) in the form of Supplementary information.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System):

The team submitting this study is one of the leading groups in the field of cancer cachexia. They have previously evaluated the genetic susceptibility for the development of cachexia syndrome in cancer patients. In the current report they were able to identify in a well-designed study that P-Selectin genotype is associated to weight loss - an important clinical feature of cachexia. Given its frequency and association with patients' quality of life and survival as well as its relative resistance to therapy any effort to identify timely and support those patients at risk is extremely important. Within this context, this piece of work is mostly welcomed.

Referee #1 (Other Remarks):

Some minor comments are:
1.A recall bias concerning the pre-morbid weight should be stated in text.
2.Since CRP is a non-specific marker of inflammation it would be necessary to know if patients with co-morbidities (i.e. infections) or those who were on medications (i.e. corticosteroids) were excluded.
3.Description of table-2 (results section) is somewhat confusing. It would be preferable for tables 2a and 2b to be described separately within text.
4.Most patients of the original cohort had primary tumors of the upper gastro-intestinal tract. Was secondary malnutrition present? Please comment.
5.More than half of the patients in the validation cohort had "other" tumor types. Are these types related with the development of cachexia and in what extend? Please comment.
6.Was P-Selectin correlated to overall survival?
Suggestion: The manuscript can be published as it is after minor revision. Not as a short report.

Referee #2 (Comments on Novelty/Model System):

Large study, well executed, dealing with an important medical problem.

Referee #2 (Other Remarks):

In M&M: please give further information on the sampling procedure.
In Results: Table 4: % of CRP data seem incorrect.
In discussion: please give some comment on the highly selected types of cancer, and on the generalisability of the data

Referee #3 (Comments on Novelty/Model System):

This well-written study examined 129 SNP's in 80 genes for their association with cancer cachexia. A strength is the fact that both a discovery and validation set of patients was used. Moreover, the investigators then looked at an animal model, induced cachexia, and observed up regulation of P-selectin.

These extra steps of validation and then utiliizing an animal model add strength to this study.

One minor suggestion is to perhaps to provide a bit more discussion on p-selectin in general.

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1st Revision - Authors' Response 07 February 2012

Please find below point-by-point responses to each of the reviewers concerns. The modifications are formatted to display the reviewer's comment first followed by the revision. The revised manuscript has been reviewed and approved by all of the authors, and as such, submitted for your timely review.

Reviewer 1:

1. A recall bias concerning the pre-morbid weight should be stated in text.

This has now been changed as suggested to:

Although there may be recall bias, evidence to support the reliability of self-reported weight and weight history is well documented (Perry et al, 1995; Stunkard & Albaum, 1981)

2. Since CRP is a non-specific marker of inflammation it would be necessary to know if patients with co-morbidities (i.e. infections) or those who were on medications (i.e. corticosteroids) were excluded.

We have now included further information in the manuscript with regards to the exclusion criteria to emphasise that patients with known infections and who were on steroids were excluded from the study

Recruitment was performed sequentially with the following exclusion criteria: i) under 18 years of age; ii) learning disability, and mental health problems; iii) inability to give written, informed consent; iv) presence of underlying infection; v) on corticosteroids

3. Description of table-2 (results section) is somewhat confusing. It would be preferable for tables 2a and 2b to be described separately within text.

This has been changed as suggested.

Table IIa lists the detailed results for SNPs significantly associated with cancer cachexia in patients classified according to weight loss alone. Table IIb lists the detailed results for SNPs significantly associated with cancer cachexia in patients classified according to weight loss with systemic inflammation (CRP >10mg/l)

4. Most patients of the original cohort had primary tumours of the upper gastro-intestinal tract. Was secondary malnutrition present? Please comment.

Indeed, cancer cachexia is considered to result from a variable combination of reduced food intake (with both primary and secondary aetiologies) and abnormal metabolism (including tumour metabolism and host inflammation) in its underlying pathophysiology. Patients with all types of cancers may have some degree of secondary malnutrition. The authors accept that this is a limitation of the study and we have acknowledged this in the discussion section.

Another limitation of the study is that patients with upper GI malignancy often report dysphagia, which may contribute to secondary malnutrition and influence the degree of weight loss. However, a previous study suggest that dysphagia may not be the sole contributing factor to weight loss in gastro-oesophageal malignancy as patients without dysphagia still report a median 4.4% weight loss at diagnosis. Moreover, in a multivariate model of the same cohort, dietary intake accounted for only 38% of variation in weight loss (Deans et al, 2009b).

5. More than half of the patients in the validation cohort had "other" tumour types. Are these types related with the development of cachexia and in what extend? Please comment.

We have added more information about the 'other' tumour types in the validation cohort and their relationship with cachexia in the manuscript as suggested.

Although, patients in the validation cohort did not have an identical distribution of cancer types as the main cohort, the distribution of BMI and weight loss remain quite similar between the two cohorts. Approximately 60% of the patients in the validation cohort had other cancer types which also had tendency to develop cachexia (e.g. prostate cancer, colorectal cancer (it is estimated that weight loss of 5% or more occurs in roughly 30% with these cancers (Dewys et al, 1980)).

6. Was P-Selectin correlated to overall survival?

We did not have sufficient data to analyse the correlation between P-selectin genotype and overall survival.

Reviewer 2

In M&M: please give further information on the sampling procedure.

More concise information on patient recruitment with exclusion criteria and data collection have now been provided in the manuscript

All subjects recruited had participated in clinical or research studies at the host institutions under ethically approved protocols, Recruitment was conducted at first presentation to surgical or oncology clinics at each institution. Recruitment was performed sequentially with the following exclusion criteria: i) under 18 years of age; ii) learning disability, and mental health problems; iii) inability to give written, informed consent; iv) presence of underlying infection; v) on corticosteroids.

Patients recruited generally had cancer types with propensity to develop cachexia (e.g. gastric/oesophageal, pancreatic, lung). Overall, 855 patients were recruited. More than 98% of the study subjects were of European descent. Information collected on each patient included date of birth, date of diagnosis, type and stage of cancer. All patients underwent measurements of height and weight at the time of recruitment to the study. Pre-morbid weight was recalled by the patient and verified where possible from the medical notes. Although there may be recall bias, evidence to support the reliability of self-reported weight and weight history (Perry et al, 1995; Stunkard &

Albaum, 1981) is well documented. Individual weight loss was calculated and expressed as percentage of pre-morbid body weight lost. Height and weight data were subsequently used to compute a common anthropometric descriptor, body mass index (BMI) (kg/m²).

In Results: Table 4: % of CRP data seem incorrect.

This has now been corrected

In discussion: please give some comment on the highly selected types of cancer, and on the generalizability of the data.

We have included a new paragraph on the selected choice of cancer types and on the generalizability of the data as suggested.

This study included a variety of cancer types, with significant numbers of patients with cancers of the digestive tract, lung and pancreas. Validation in larger independent cohorts is required to fully establish the generalizability of our findings, however the significant association with the rs6136 polymorphism and cachexia across both our main group and an independent validation cohort suggest that our results may apply across numerous cancer types.

Reviewer 3

One minor suggestion is to perhaps to provide a bit more discussion on p-selectin in general

More information about P-selectin has been included in the discussion section as suggested

The human P-selectin gene spans over 50 kilo-base-pairs on chromosome 1, containing 17 exons, almost all of which encode distinctive domain structures (Johnston et al, 1990; Watson et al, 1990). It has both membrane and soluble forms in platelets and endothelial cells (Johnston et al, 1990). Both the membrane and soluble forms of P-selectin bind to leukocytes. In certain inflammatory conditions, the plasma concentrations of soluble P-selectin is highly elevated. (Dunlop et al, 1992). It is suggested, that the membrane and soluble forms of P-selectin may work co-ordinately in vivo for the regulation of their cell adhesion and, perhaps, signalling functionality. P-selectin has been characterized previously by approaches such as gene knockout or the use of specific inhibitors to be involved in the recruitment of neutrophils and macrophages in inflammatory responses (Borges et al, 1997; Chen & Geng, 2006). P-selectin may also participate in intra-tumoural regulation of the genesis of systemic inflammation via the innate immune system and/or regulation of the complex interaction within muscle between the endothelium and signalling pathways in muscle fibres (Wagenmakers et al, 2006).

