Author's response to reviews

Title: Identification of novel functional sequence variants in the gene for peptidase inhibitor 3

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Dr. Melissa Norton, The Editor,
BMC Medical Genetics

RE: MS 1746572592964553

Dear Dr. Norton,

Thank you for the review of our manuscript titled “Identification of novel functional sequence variants in the gene for peptidase inhibitor 3.” We have carefully read the reviewers’ comments and thank them for their time, effort and constructive criticism.

General.
During our revisions we have replaced the term “fetoplacental membranes” with the preferred term “chorioamniotic membranes.”

No response is required for Dr. Niels Uldbjerg.

Our responses to Dr. Felipe Vadillo-Ortega are:

1. Functional effects on the transcription of PI3 gene associated to the two SNPs and relevant to the amnion biology were not addressed in this paper, and hence there is not experimental evidence to support the assumption of a relation between these variants of the gene and an effect on downregulation of PI3 gene in amniochorion affected by PPROM.

We have added the following “We have performed a genetic association study with PI3 variants, including the –689C>G variant, and found that it is associated with PPROM [manuscript in preparation]. We also previously demonstrated by immunohistochemistry that many cell types of the chorioamniotic membranes produce PI3 and that PI3 protein is decreased in chorioamniotic membranes from PPROM cases[1]. Together, these lines of evidence…” to the Discussion to indicate some unpublished results (manuscript in preparation) derived from a large genetic association study. The relevant result is that the –689C>G variant is associated with PPROM. This additional evidence strengthens the original statement that we think that the evidence in this manuscript together with the one in preparation and the prior publication[2], “provide a plausible genetic explanation for the down regulation of PI3 in chorioamniotic membranes from PPROM cases.”

2. More information on the characteristics of the membranes used to derive amnion primary culture in this study must be provided, in order to figure out the possible role of tissue functional status related to the presence/absence of labour and expression of the nuclear factors that recognize both SNPs.

We have added “…since we had previously demonstrated that PI3 protein was produced by a variety of chorioamniotic membrane cell types with the highest amount produced by the amniotic epithelial cells [1]. All chorioamniotic membranes used to culture amniotic cells were from women at term who underwent cesarean sections; consequently the cells were not exposed
to labor.” to the Methods section under the subheading “Electrophoretic mobility shift assays (EMSA).”

Discretionary Revisions:
The general hypothesis behind these studies is related to an altered proteolysis balance in
the amniochorion conducting to PPROM. Despite it is not the purpose of this paper, I would
like to raise some question for this hypothesis that can be commented by the authors: How
relevant is an elastase for connective tissue integrity in amniochorion? Is elastase actually
transferred from the amniotic fluid to the amniochorion extracellular matrix? Is the chorion a
source for this peptidase inhibitor?

There are three separate questions in the comment.
1. How relevant is an elastase for connective tissue integrity in amniochorion?

This is an excellent question that is difficult to address in the current manuscript. The answer to
this question has not been answered experimentally. There is evidence that elastase and the
secretory leukocyte peptidase inhibitor (SLPI) accumulate in the amniotic fluid[3] and also that
the concentration of elastase and SLPI are highest at the site of rupture of the chorioamniotic
membranes [4]. In part, the question is related to the specificity of elastase. It has been shown
that human neutrophil elastase (ELA2) can degrade a number of extracellular matrix proteins
including proteoglycans, collagens, including basement membrane collagens, and fibronectin. It
has also been show to digest cell surface receptors such as CD14 [5] and to be involved in
degrading/destabilizing adherens junctions[6]. There is also some evidence that ELA2 can alter
the permeability of epithelial/endothelial cells [7, 8], an observation that may explain the strong
staining for both PI3[2] and SLPI[4] in the amniotic epithelial cells, Thus, there are inferential
data to suggest that it may play a role in degrading the chorioamniotic membranes. The question
needs, however, to be addressed experimentally.

2. Is elastase actually transferred from the amniotic fluid to the amniochorion extracellular
matrix?

Yet another excellent questions that has not been addressed experimentally and therefore the
answer is unknown. The observation of increased ELA2 elastase at the site of rupture compared
to the rest of the chorioamniotic membrane suggests that there may be a local source. In the case
of PPROM, however, high levels of elastase in the amniotic fluid may alter the permeability of
the epithelium [7, 8] to allow transfer of elastase to the chorioamniotic extracellular matrix. This
question may be addressable in animal models of amniotic inflammation.

3. Is the chorion a source for this peptidase inhibitor?

We have previously shown that there is staining for PI3 in or around intermediate trophoblasts.
Thus PI3 is present in the chorion although it has not been definitively demonstrated that it is
produced in the chorion.
In both the mandatory revisions above, we have indicated that multiple cell types in the chorioamniotic membranes produce PI3. Although it is not possible to directly address the question regarding the relevance of elastase, the production of PI3 by numerous cell types of the chorioamniotic membranes suggests that it has a functional role. Whether that is in relation to elastase or not can only be addressed by other experiments.

Yours Sincerely,

Gerard Tromp