Author's response to reviews

Title: HFE Gene Variants Modify the Association between Maternal Lead Burden and Infant Birthweight: A Prospective Birth Cohort Study in Mexico City, Mexico

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Author's response to reviews: see over
To the Editor,

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“HFE Gene Variants Modify the Association between Maternal Lead Burden and Infant Birth Weight: A Prospective Birth Cohort Study in Mexico City, Mexico”

On behalf of the authorship team we thank the reviewers for their comments. Please see our point by point response below, beginning with reviewer 1. All corresponding responses have been altered in the revised manuscript using track changes. Please don’t hesitate to contact the primary author for any clarifications.

Again we thank everyone for their comments and we hope all concerns have been addressed.

Reviewer 1: (Pam Factor-Litvak)

Minor essential revisions:

1. Please clarify the results in table 5. In particular, the estimated coefficient between infant wildtype/maternal variant and birth weight is given as +143.0 grams.

We have updated both the table and the results section to better clarify what both tables 4 and 5 are showing. In table 4 we are examining the main maternal and infant gene effects of HFE and TF variants upon birthweight, while in table 5 we are examining how these genes may modify the adverse association between lead exposure and birthweight.

In summary, table 4 indicates that infant H63D variants have an adverse association with birthweight, while the maternal H63D variants appear to have no association.

The main gene estimated coefficient between infant wildtype/maternal variant and birth weight given in table 5 (model 5) as +143.0 grams and the interaction term (-28.7) suggests that while maternal H63D variants may not directly impact birthweight, they may enhance lead’s adverse association with birthweight. It is interesting to note that when comparing the estimated coefficient from model 3(-62.7grams) and both the estimated coefficients for infant wildtype/maternal variant (+143.0 grams) and infant variant/maternal variant (-177.2 grams) in
model 5 that any direct effect upon birthweight by H63D gene appears to be driven by infant H63D variants.

2. Some explanation of how the maternal variant/infant variant interaction would biologically work is needed. Are there other examples of maternal - infant gene interactions?

We thank the reviewer for this comment since one interesting aspect to this pilot study is that a majority of epidemiological birth cohort studies rarely consider the combined effects of maternal and fetal genotype. By no means is this study setting precedence (Liang et al. 2010; Qin et al. 2008; Relton et al. 2004; Tan et al. 2009) and we completely acknowledge that our study is underpowered, but feel that these pilot results and previous work from other researchers provide compelling evidence that a closer look at maternal-fetal gene interactions is needed in future studies. We provided an additional paragraph in the discussion summarizing past maternal-fetal genetic interaction studies and emphasizing the importance of looking at this association in the future.

In response to an explanation of how maternal/infant variant-variant interaction would biologically work.

In our discussion we proposed potential biological pathways for our findings for the association of the maternal H63D polymorphism in enhancing lead’s adverse effects upon birthweight and the infant H63D polymorphism association with decreased birthweight (paragraphs 3&4 in the discussion.)

While it is interesting to hypothesize that if both mother and infant carried the variant the subsequent effect upon birthweight might be a result of the combined potential biological pathways, something akin to Knudson’s “double-hit” hypothesis in cancer literature (Knudson AG, 1971), our study doesn’t have the sufficient power or is correctly designed to elucidate potential mechanisms for these maternal-fetal gene-gene-environment interactions in depth.

We did add a sentence in our discussion “We speculate that these effects could be enhanced when both the mother and infant carry the variant allele potentially due to a similar mechanistic pathway.”

In speculation (and we emphasize speculation): maternal H63D variant carriers may have increased lead absorption throughout pregnancy, especially during iron deficient conditions (though this has never been directly studied during pregnancy), creating a larger available pool of lead to cross (and directly damage) the placenta. In addition infant H63D variant carriers may have more available DMT-1 transporters on the placenta to allow lead to cross. Unfortunately there haven’t been any focused mechanistic studies observing how increased expression of
DMT-1 transporters may impact the transfer of lead across the placenta under both adequate and extremes in iron levels.


3. Another possible explanation for the hemoglobin finding (particularly since better measures of iron status and iron metabolism were not obtained) is that lower hemoglobin concentration is also associated with insufficient oxygenation of the blood, including blood going to the placenta (and the fetus). Please comment.

We agree that many researchers have indicated that in response to low iron and anemic conditions in early (1st trimester) and even late (3rd trimester) pregnancy changes in placental morphology occur. These changes manifest as larger weigh placentas with observed increases in villous volume and surface areas of the capillaries involved in gas exchange which thought to be in response to hypoxic conditions that would limit growth (Hindmarsh et al. 2000; Howe et al. 1995; Kadyrov et al. 1998; Singla et al. 1997.) Interestingly, in all our models increasing levels of maternal hemoglobin at 1 month postpartum were actually associated with a decrease in birthweight which suggests a mechanism more similar to iron excess. Though based on comments from both reviewers, and our own acknowledgement, that utilization of 1 month post-
partum hemoglobin levels are not a good reflection of iron status during pregnancy (therefore we cannot assess iron excess or deficiency during pregnancy) we hesitate to include a potential explanation for this secondary finding. Instead we chose to include the reviewer suggested mechanism as an explanation for the association between anemia and low birthweight.


Reviewer 2: (Fernando E. E Viteri)

The major weakness of this paper is the fact that the authors do not account for the possibility of the effect of maternal iron status prior to and during pregnancy, including the ingestion of iron from antenatal supplementation.

We agree with reviewer 2 that this is a significant limitation. Unfortunately, due to the original study design individuals were recruited at delivery and not during or prior to pregnancy. We acknowledged this limitation in our discussion.

In essence, the lack of information on maternal iron status and supplementation during pregnancy introduces a serious confounding factor on the relation between HFG mutations and birth weight.

Again we agree with reviewer 2 that with a lack in markers of maternal iron status during pregnancy, since the original study design recruited women during pregnancy, we do not have the ability to assess whether iron status is a potential confounder or mediator in the pathway of HFE effects upon birthweight. We have future plans to address this topic in more depth with a different cohort.

Another confounding factor that is not properly addressed in this paper is the relation between calcium intake and possible bone release of lead.

Again we agree with reviewer 2 that calcium intake during pregnancy can influence release of bone lead stores into the body. Unfortunately, as pointed out by reviewer 2 the original aim of the project was to determine the effect of calcium supplementation on bone lead mobilization during lactation post partum and as such we were unable to properly determine calcium intake and supplementation during pregnancy.

Are there data available on maternal blood lead besides bone lead?
We updated Table 3 to include the main effects of maternal blood at delivery upon birthweight.
We also included a sentence in the last paragraph of the Results section which states “There were no significant interactions between maternal blood at delivery and genotype status (data not shown.) “

Are they significant covariates?
Maternal blood lead at delivery was not a significant covariate in any of our models.

Even though the homozygous HFE H63D and compound heterozygotes were removed for good statistical reasons, their results should have been presented given that they could have been “outliers”.
We thank the reviewer for this insight. The compound heterozygotes were originally removed for biological reasons since several large studies have indicated serum ferritin, transferrin saturation, and hemoglobin levels are significantly increased in this individuals when compared to wildtype individuals (Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, Napier JA, Worwood M: HFE mutations, iron deficiency and overload in 10,500 blood donors. Br J Haematol 2001, 114:474-484; Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marchesini G, Fargion S: HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2010 Mar;138:905-12.)

We altered the statistical analysis in our methods section to indicate in this population we have N=1 compound heterozygote mothers and N=2 compound heterozygote infants (as well as N=3 maternal and N=4 infant homozygous individuals.) When these individuals and those individuals homozygous for the HFE H63D genotype were included as “carriers” (i.e. were recoded HFE = 1 versus HFE =0 for wildtype) the direction of the effect estimates were the same but the main effect of both the infant and maternal genotype were slightly attenuated. We included the updated effect estimates in the results section text.

Lastly, some minor editorial corrections or modifications could be suggested:
Indicate that it is maternal bone lead every time bone lead is referred to.
Completed, see track changes.

Wild type HFE H63 and not HFE H63D should be indicated as homozygous nonvariant.
The same should be for the homozygous wild type genes C282 and TFP570.
Completed, see track changes.

Thank you in advance for your time and consideration. I will be the primary author for correspondence.

Sincerely,

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