Author's response to reviews

Title: 'Fat mass and obesity associated' gene (FTO): no significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents

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Author's response to reviews: see over
Dear Editor,

please find enclosed the revised version of our manuscript MS: 9850512901856888 - 'Fat mass and obesity associated’ gene (FTO): no significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents.

We thank the reviewers for their very positive and constructive comments. In the revised manuscript and in our answer, we addressed the suggested changes according to the reviewers' recommendations.

Reviewer 1 (Hakon Hakonarson)

1. Is this the same study cohort that the investigators recently reported association on in PLoS One – (at least 85 of them that had their ARMS-PCR genotypes confirmed through GWA appear to be): If this is the case this is not an independent replication of association of this FTO SNP with obesity and should be references/clarified as such.

We here report an association of the FTO SNP rs9939609 with early onset obesity in a case-control study comprising 519 German obese children and adolescents and 178 normal weight adults (p=0.036). None of the individuals reported in our recently published GWA (Hinney et al., PLoS ONE 2007) has been used in the current analysis. We hence report an independent association of this FTO variant with obesity. On page 5, line 14-15 we now stated that all individuals used in this study were independent of our GWA.

We apologize for confusing the reviewer with the inclusion of 85 individuals of our GWA for the establishment of the ARMS-PCR. These 85 individuals were not included in the current case-control study but solely used for control purposes. Analyses of these individuals were performed to ensure validity of genotyping and to address potential problems of genotype calling algorithms as described in McCarthy et al. (2008). On page 6, line 17-18 we now stated that none of the 85 individuals were incorporated in the association analysis.

2. The control group examined is small (n=178) so the study is underpowered to conclude on lack of association; the authors provide power calculations for multiplicative genotype relative risk model (and regression based analyses) which may not be correct model. The OR for association with obesity is 1.24 suggesting the effects they are looking for in relation with other traits are unlikely to be higher.

As we were able to confirm the association of the analysed FTO variant with obesity in this study (p=0.036; OR_{AT} 1.24; 95% CI 0.98-1.57; OR_{AA} 1.54, 95% CI 0.96-2.46) the power concern is of minor importance as power issues usually arise in case of non-significant findings. Nevertheless to address possible power concerns and to check the robustness of the result, we additionally performed a sensitivity analysis including the 442 underweight individuals from our recently published GWA (p=5.70x10^{-6}; OR_{AT} 1.47, 95% CI 1.24-1.73; OR_{AA} 2.15, 95% CI 1.54-2.98) in an exploratory case-control comparison. This latter analysis, however, is not independent of our GWA finding which has now been pointed out on page 8, line 4-6.

With regard to the other traits, we agree that the power estimates might be lower due to even smaller effect sizes as e.g. indicated by a very recently published study (Freathy et al. Diabetes 2008;57:1419-1426). This point has now clearly been pointed out on page 9 line 11-14. The respective reference has been stated.

3. The authors state that the degree of overweight was quantified with Cole’s least mean square method, which normalized the BMI skewed distribution in childhood and expressed BMI as a standard deviation score (BMI-SDS). The authors should clarify how this method compares with perhaps better standardized definition of z-score by Center for Disease control (CDC).

The idea behind using BMI z-scores and BMI-SDS is basically the same; a larger sample is used to standardize/transform the new data with regard to age and sex to standard normal distribution such
that units are given in units of standard deviations assuming that a normal distribution correctly models BMI or by using a transformation to make the sample data more normally distributed (Cole TJ. Eur J Clin Nutr. 44: 45-60, 1990) which better takes into account the skewness of the BMI distribution. In either case 1 BMI-SDS means that the individual is approximately 1 standard deviation above the mean of his/her age- and sex matched population mean. On page 4 line 24 and on page 5 line 1 we now stated that the BMI-SDS is a comparable measure to the standardized definition of the BMI z-score as e.g. used by the Center for Disease Control (CDC) if BMI is normally distributed in the population.

4. Are all the study subjects Caucasians? It may be relevant to refer to the recent pediatric study reported on the association of a common tagging SNP in both Caucasians and African-Americans (Grant et al. PLoS ONE, 2008).

On page 4, line 18-19 we now stated that all individuals used in this study were of European descent.

4. The controls used include lean adults mean age 24 years compared to mean age of 10 years for the cases – where the adults overweight as children?

The reason for choosing older normal weight individuals as controls is that these are less likely to develop obesity later in life; hence the genetic difference between these two groups should be higher than for normal weight children and adolescents. The analyzed children and adolescents are extremely obese, so that their risk of staying obese is very high. To underline this difference between the groups we added the term 'normal weight adults' to describe the controls.

Reviewer 2 (Liselotte Petersen)

1. There are two well-defined and interesting questions posted in the paper, both relevant and will contribute new knowledge to the field. One, the most important, is whether FTO is associated with weight loss during an intervention study; the other, if FTO is associated with blood lipids in obese children at baseline. These two questions are the main issues, which are apparent in the title and the abstract. I think the paper would gain much if these issues were also the main focus of the rest of the paper. It seems to me that the confirmation part of FTO and obesity gets to much focus. In my opinion, the confirmation analysis is not underscoring anything; it is mostly relevant as a check of the data.

We agree that the test for association with obesity status in the case-control approach was only relevant to check whether it is worthwhile to analyse the implication of the rs9939609 genotype status on weight loss and lipid markers in this group of obese patients. We do also agree that the novel findings should be more focussed and restructured the text accordingly.

2. Where was the control group recruited? I think a comment is lacking about the age distribution, which is completely different from the group of obese children.

On page 5, line 12-14 we now included where the normal weight individuals have been recruited. The reason for choosing older individuals as controls is explained in the answer to a similar question of reviewer 1 (see above).

3. To be comparable with the rest of the obesity group, the fasting blood parameters are baseline measurements for the intervention group, right? This should be clear from the text (and in the table).

The delta BMI analyses should more clearly be distinct from the baseline analyses in the tables as well as in the text.

The fasting blood parameters are baseline measurements of those obese individuals where the measurements were available. A respective explanation can now be found on page 6, line 21 as well as in table 3. Additionally, we now clearly separated the longitudinal data (table 2) from the cross-sectional data (table 3) and included the total number of individuals for each variable in the tables.
4. The analysis of BMI-SDS is at baseline too? So the association to obesity in the case-control setting is not found in degree of obesity within a group of obese. I suggest to point this out in the discussion.

The mentioned BMI-SDS values are indeed baseline measurement which has now been underscored in table 2. We now point this out on page 8, line 11-14) and explain that the observed association with obesity is not found for the degree of obesity within the group of obese individuals.

5. To avoid confounding from regression to the mean, the delta BMI-SDS should be adjusted for baseline BMI, though this might be an over-adjustment, because it can also be seen as part of the pathway. At least a comment on this issue would be appropriate. In the analyses, is there any confounding from gender?

This is an important point raised by the reviewer. We did additional robustness analyses including baseline BMI-SDS in a regression model. This analyses had almost no impact on the estimator under the additive genetic model; it was -0.031 (95% CI -0.090…0.027) compared to -0.032 (95% CI -0.091…0.027) reported in the paper (note that the upper CI bound did not change due to rounding). The main effect of sex within both regression analyses had a p-value >.7 not indicating major gender differences in delta BMI-SDS. A respective footnote was added to table 2.

7. Is the adjustment for age linearly? Is that appropriate in all analyses?

Yes, age was modelled as linear predictor. To check this assumption we plotted age against the other variables. From this plot there was either no relationship at all or no other relationship deducible. Consequently, we also checked the model omitting age entirely which again did not have a notable impact on the reported genetic estimates (within the range of rounding errors).

8. A couple of places it says ´extreme early onset obesity´ do you mean ´early onset extreme obesity´?

We have changed ´extreme early onset obesity´ to ´early onset extreme obesity´.

9. A few places it says Fto, instead of FTO

The abbreviation FTO is used for the human ´fat mass and obesity associated´ gene whereas Fto describes ´fat mass and obesity associated´ gene in non-human individuals.

Reviewer 3 (Emanuele Miraglia del Guidice)

1. Please identify the statistical software package used to analyse the data.

The respective software packages are now stated on page 7, line 16-17.

2. One of the problems with any dietary program is compliance. The authors did not mention any approaches to tackle this problem nor did they mention any recording of the subject’s food intake. Also physical activity was not taken into account.

All individuals who participated in the intervention program had to prove their motivation by filling out a questionnaire concerning their eating and exercise habits and by attending exercise groups for overweight children regularly for at least 8 weeks. Only children who had filled out the questionnaires and who had participated in the exercise groups were included in the ‘Obeldicks´ lifestyle intervention program independently of what they have stated in the questionnaires. This point is now clearly pointed out on page 5, line 19-24. Food intake was not measured in our study, as description of food intake underlies a remarkable bias due to the patients’ tendency of underreporting this behaviour.
Formatting changes

1. Abstract - Please could you structure your abstract according to the instructions for MEDICAL manuscripts (add a Methods section)

A `Methods section` has now been included in the abstract.

2. Competing interests - Please include a `Competing interests` section between the Conclusions and Authors' contributions.

A `Competing interests` section has now been included.

3. Authors' contributions - Please include an Authors' contributions section before the Acknowledgements and Reference list.

An `Authors` contributions` section has now been included

4. Please ensure that your revised manuscript conforms to the journal style

The format of the revised manuscript has been changed accordingly