Author's response to reviews

Title: Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS): a case control study

Authors:

Susanne Tan (susanne.tan@uk-essen.de)
André Scherag (andre.scherag@uk-essen.de)
Onno Eilard Janssen (onno@onnojanssen.de)
Susanne Hahn (mail@susannehahn.com)
Harald Lahner (harald.lahner@uni-due.de)
Tiina Dietz (tiina.dietz@uk-essen.de)
Susann Friedel (susann.friedel@uni-duisburg-essen.de)
Harald Grallert (harald.grallert@helmholtz-muenchen.de)
Carla IG Vogel (carla.vogel@uni-due.de)
Rainer Kimmig (rainer.kimmig@uk-essen.de)
Thomas Illig (illig@helmholtz-muenchen.de)
Klaus Mann (klaus.mann@uk-essen.de)
Johannes Hebebrand (johannes.hebebran@uk-essen.de)
Anke Hinney (anke.hinney@uni-due.de)

Version: 2 Date: 20 September 2009

Author's response to reviews: see over
Revision of the manuscript 1721762553281595 by Tan et al.

Dear Editor,

please find enclosed our revised manuscript 1721762553281595 titled ‘Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS)’ by Tan et al. for BMC Med Genet.

We thank the reviewers for their extremely valuable comments and the constructive criticism on the previous version of our manuscript. On the following pages we responded to each criticism and indicated the changes that we have made.

We thank you for re-considering our manuscript for publication in BMC Med Genet.

Sincerely,

PD Dr. Anke Hinney

Department of Child and Adolescent Psychiatry
University of Duisburg-Essen
Virchowstr. 174
45147 Essen
Germany
Phone: +49-201-7227 251
Fax: +49-201-7227 302
Email: anke.hinney@uni-due.de
Reviewer 1 (Thomas Barber)

We thank the reviewer for his valuable comments. In the revised manuscript and in this letter we have addressed all concerns.

Major Compulsory Revisions

1. My main concern relates to the number of analyses performed in this study. The authors genotyped SNPs within four separate genes and performed numerous analyses on each one including correlations with various metabolic and endocrine phenotypes. Although there is mention of Bonferroni-adjustment in the methods, I feel that there could be more mention of this in the results section. Furthermore, the high number of analyses performed could be mentioned as a limitation of the study in the discussion section. It may be worth considering statistical review of the manuscript for this reason.

This concern has also been raised by reviewer 3. Thus, we have applied an advanced method for multiple testing to control for all statistical tests performed (Conneely and Boehnke (2007): So many correlated tests, so little time! Rapid adjustment of p-values for multiple correlated tests. American Journal of Human Genetics 81:1158-1168.) We have added the reference to the Material and Methods section (page 7) and have discussed the results (page 9).

2. I do not fully agree with one of the conclusions that FTO variants may have a greater effect on BMI in women with PCOS than in the general population. I believe that there may be alternative explanations to this apparent finding that should be discussed further by the authors in their manuscript. The numbers of cases and controls in this study were disparate which would presumably have resulted in differences of power to detect effects on BMI between each of the groups. Furthermore, figure 1 demonstrates a larger distribution of BMI amongst the PCOS group than amongst the controls. These two factors may offer an alternative explanation to the apparent larger effect of FTO variants on BMI within the PCOS group than among controls. Only through studying a much larger population of PCOS cases would the true effect-size of FTO variants on BMI in this group become apparent.

We agree with the reviewer that including a larger sample of PCOS cases would indeed be beneficial. An increase in sample size would only have an effect on the precision of the estimator by reducing the standard error (assuming the effect is true). Another issue is the variance of BMI in PCOS cases as compared to population-based controls. Based on our data, the variance of BMI in PCOS cases seems to be larger than the variance in population-based controls of the same age range. Again, an increase in variance will only have an impact on the precision of the effect size estimator given that variance and effect are independent which we do not know. Thus, we used robust linear regression models (see page 7), which are less sensitive to extreme values than standard linear regression models. In addition, we refer to the literature which confirms our observation (see page 9).

3. In the methods section, it is stated that there are 386 PCOS cases, all of whom satisfied Rotterdam criteria. It would be useful to include the numbers of PCOS cases that satisfied all three criteria, and numbers of those that satisfied only two criteria. It is well-established that women who satisfy all three Rotterdam criteria are significantly more insulin-resistant than those women with polycystic ovaries who only have one of hyperandrogenism or oligo-amenorrhoea (see Barber et al. Clinical Endocrinology [2007], 66, 513-517). Therefore, in the context of a study that assesses the effects of gene variants on insulin resistance in PCOS, it would seem relevant to include details on Rotterdam phenotypic subgroups.

A descriptive phenotypic subdivision of the PCOS cases has been added to the result section (see page 8 and table 1). However, addressing the problem of even more statistical tests, we decided not to perform additional subgroup tests comparing genotype distributions.
Minor Essential Revisions

1. Abstract: it would be useful to give a mention of the control group.

Description of the control group has been added to the Material and Methods section of the abstract.

2. Background, 1st paragraph, 3rd sentence: This sentence implies that the association of PCOS with obesity is secondary to the association of PCOS with insulin resistance, which is misleading. Rather, it should be made clear that there exists an association between PCOS and obesity, and between PCOS and insulin resistance. The relationships between these factors are not completely understood, but it is reasonable to postulate that insulin resistance in PCOS, at least in part results from the association of PCOS with obesity. There is also evidence that insulin resistance in PCOS plays an important role in its aetiology (through, for example effects of hyperinsulinaemia on ovarian steroidogenesis).

The 3rd sentence of the 1st paragraph of the background section is indeed misleading and has been corrected (see page 3).

3. Results: From figure 1, it appears that data for BMI was available for the controls. Were any other phenotypic data available for the control group? It would be interesting to show analyses on the effects of genotype variants on BMI and other phenotypic variants

Unfortunately, we only had access to the BMI phenotype for this particular analysis. Other phenotypes are available for KORA (http://www.helmholtz-muenchen.de/epi/beitraege-zu-netzwerken/kora-gen/index.html) but they did not match to those obtained for our PCOS cases.

4. Results: I was surprised that the variant within TCF7L2 had an effect on BMI within the PCOS cases group. As the authors correctly state, the likely effect of variants within TCF7L2 on risk of T2D is through effects on the beta-cell. Would the authors like to speculate on a possible mechanism by which variants in TCF7L2 affect BMI in PCOS cases? Have any other previous studies demonstrated such an effect on BMI?

Does a similar effect of TCF7L2 variants on BMI exist in the control group?

The discussion (page 10) referring to our TCF7L2 results has been extended including previous reports showing a BMI correlation. Exploring the effect of TCF7L2 variants (rs4506565 as proxy for rs7903146 with an r²=0.917) on BMI in a subset of 831 females (n=175 in the same age range as the PCOS patients) for which we had access to 500k genome wide association study data yielded no evidence of differences to the PCOS cases (in KORA the per (minor) allele effect was β=0.05; p=0.83 in the 831 females and β=-0.14; p=0.79 in the subgroup of n=175).

Quality of written English

Needs some language corrections before being published

We checked and corrected the manuscript carefully.
Reviewer 2 (Alessandra Gambineri)

We thank the reviewer for her valuable comments. In the revised manuscript and in this letter we have addressed her concern.

I have only a major concern that is the needed for an age- and BMI-matched group of controls. This is mandatory to accept the conclusions given by the Authors. Therefore, the study needs for this major revision.

We thank the reviewer for addressing this topic. Indeed the title of our study must have been a little confusing as our study is not a classical case-control study. Consequently, we changed the title to “Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS)”. As basic study layout we investigated known genetic variants, confirmed by evidence from genome-wide association studies, in a sample of PCOS patients focussing on associations of the variants with metabolic / obesity related variables, variables of IR, PCOS symptoms and variables of hyperandrogenemia. Based on the observed large BMI effect of FTO variants in our PCOS cases which has been observed before (see page 8), we used a population-based control group of about 2,000 females (KORA study: Kooperative Gesundheitsforschung im Raum Augsburg, Survey 4) of the same age range as the patients with PCOS to support our observation. In this analysis, BMI was the outcome and age was controlled for by limiting the analyses to the same age range and by applying a robust multiple regression model. This procedure has now been described in detail on page 7.
Reviewer 3 (Margrit Urbanek):

Major Compulsory Revisions

1. Since all the genes studied here are candidates based on previous genetic studies and the PCOS cohort sample size is quite small it is essential that the authors provide the appropriate power calculations which in this case would be the power to detect effect sizes comparable to what has been observed in the literature for either PCOS or if that is not available for T2D.

In the revised version of the paper we have extended our power considerations referring to effect size estimators from the literature (see page 7-8).

2. Only one finding remains statistically significant after correction for multiple test (24 for tests; arguably the correction should be more stringent since there were as many as 14 phenotypes). This needs to be made clear to readers or the results need to be replicated in an independent cohort.

We have applied an advanced method for multiple testing to all statistical tests performed (Conneely, K.N. and Boehnke, M. (2007) So many correlated tests, so little time! Rapid adjustment of p-values for multiple correlated tests. American Journal of Human Genetics 81:1158-1168). We have added the reference to the Material and Methods section (see page 7) and have discussed the results (see page 8).

Thank you for your time and effort in evaluating our manuscript.

Yours sincerely,

PD Dr. Anke Hinney