Reviewer’s report

Title: Genetic variations in APPL2 are associated with overweight and obesity in a Chinese population with normal glucose tolerance

Version: 2 Date: 19 January 2012

Reviewer: Francis Vasseur

Reviewer’s report:

The manuscript entitled Genetic variations in APPL2 are associated with overweight and obesity in a Chinese population with normal glucose tolerance by Shan Jiang et al. has been greatly amended and thus greatly improved. Moreover many additional investigations requested have been performed.

All the initial remarks from #1 to #7 ; #12 to #17, #19 and #20 have been taken into account.

Some previous remarks still need clarifications.

Minor essential revisions

#a : In the table 1 the authors should avoid the SE for « age » and prefer to present the standard deviation SD.

#b : In the legend of table 4 the authors wrote « Log transformed values were used for p, Beta and SE values ». Do they mean that the obesity related phenotype under investigation was log transformed before use in the GLM ? If so it would be clarified in the legend of the table 4.

Major compulsory revisions

#c : Following the previous #9 remark and the authors answers, some questions and remarks must still be drawn. Regarding the GLM analysis of the whole set of subjects investigating the putative link between rs2272495 and obesity related phenotypes it is surprising that when stratifying according to the 3 groups : « normal » « overweighed » and « obese », none analysis was significant. Indeed when focusing on the most significant phenotype (BMI) as reported in the present version Table 4 (with a 0.008 pvalue), the median BMI values are almost equal across the 3 genotypes in the normal weighted population (21.702 ;21.713 ;21.770 for the TT ;CT ;CC), likewise in the overweighed population (25.637 ;25.467 ;25.646). Thus with such « equal » median values across the genotypes and the quite unsignificant pvalues (0.413 ;0.497) that do no reflect even a trend toward association, at least in these two subgroups of patients it an association between BMI and rs2272495 that would not reach significance because of a lack of power when stratifying is questionable. A similar conclusion may be drawn for the obese group as mean BMI values are similar across genotypes and pvalue is unambiguously not significant. Indeed inside each group, there is no evidence suggesting increasing BMI according to genotypes and each strata appears homogeneous according to the obesity related phenotype under study even if this phenotype differs between strata. Thus it appears that the associations with
obesity related phenotypes and rs2272495 are only the reflect of the association between rs2272495 and overweight, and between rs2272495 and obesity as it is reported in table 2. According to the data presented in the manuscript it is difficult conclude to an association with obesity related phenotypes but only of an association with overweight and with obesity. Does the distribution of obesity related phenotypes is under a continuous (even skewed) distribution or displays 3 groups ?). The homogeneous obesity related phenotypes values inside strata plead for a 3 groups distribution. It is likely that when using all participants this whole population is indeed stratified in 3 groups that may not be mixed into a statistical analysis.

#d : answering to #11 the authors think that the GLM remains more suitable for their data. There is no reason to reject the GLM procedure but it was only suggested to use this GLM procedure in a non-parametric context using the Conover and Iman method. Unfortunately although the authors claim they see increasing values of (i.e.) BMI according to the number of C alleles most of the Dunnet contrast tests they report were unsignificant. Thus it is impossible to conclude to an additive model and the hypothesis of a recessive model as pointed out by my previous estimated calculation remains a possibility that the authors should have tested. However referring to #c the results do not allow to conclude to an association with obesity related phenotypes and thus the manuscript should be amended accordingly.

#e : Regarding the answer to #18 although the multivariable linear regression model the authors used was performed under an additive model, a significant result does not implies that biologically it is an additive model according to the presence of a given allele. Moreover as association strongly suggests a recessive model (see #11 of the previous report) and as the Dunnet contrast tests they report were unsignificant and do not allow the authors to conclude to a given genetic model, the affirmation by the authors of an undemonstrated additive (co-dominant) model remains an over interpretation of the data.

Some new suggestions arise from the new amended version of the manuscript.

Major revision

#f : The sentence at line 153 « carriers of a greater number of rs2272495 C allele exhibited higher WHR » that refers to table 4 is an over interpretation of the data as median WHR is 0.849 for TT and 0.849 for CT, thus WHR is not higher in patients having one C allele as compared with those having none.

Minor revisions

#g : As the authors now pooled overweighted and obese patients, as they did at line 144 it would be more convenient to present all the results as : « …evidence of associations with overweight/obesity… » rather than « …evidence of associations with overweight and obesity… ». 

#h : at line 200 the authors should use « underlying » and at line 206 « Bonferoni ».

#i : at line 208 the sentence may be misunderstood : one may think that the authors tempted a replication that was negative. They should write that they had
not the opportunity to perform a replication another independent sample.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests