Reviewer’s report

Title: Association between a rare SNP in the second intron of human Agouti related protein gene and increased BMI

Version: 3 Date: 5 March 2009

Reviewer: Yvon Chagnon

Reviewer’s report:

1. Referee’s comment: “Three SNPs have been detected in this sample, and the authors choose to study the less variable of them for which no variant homozygote was observed. Why they did not analyzed also one of the two other SNP that are in complete linkage disequilibrium with each other?”
   It would be important to analyze SNPs that have been reported previously to be associated with body composition such as Ala67Thr. Did the authors detect this relatively frequent variant in their sample of 95 patients?

1. Answer: The rs5030980, with MAF=0.045, was found in 95 sequenced patients and corresponds to Ala67Thr as described in the “introduction” and “results and discussion” sections.
   This is true. But since data are already available in their cohort, and since this is the only positive association reported previously, it is very strange that the authors did not present these results. As a reader, I would like to know what happen in this cohort and how these results contrast with the new SNP.

   This particular association analysis was performed to evaluate the role of a rare SNP’s found in resequencing of specific cohorts of patients. We feel that analysis of rare SNP’s are often underrepresented compared to common SNP’s, while the combination of rare SNP’s may have great impact on the genetics of common diseases. Although we agree that replication studies are of great importance, the main goal of this study was to analyse SNP that was not previously studied with respect to obesity.

   But why not present these results or at least made a statement on them in the Discussion?

2. Referee’s comment: “In the Abstract, what is the meaning of “carriers of the rs11575892”? All subjects carried this SNP. Also, the authors could not conclude that they reported for the first time an association of AGRP with BMI, two previous reports made it earlier, Argyropoulos (2002) with late-onset obesity, and Marks (2004) with different body composition phenotypes.”
2. Answer To avoid misunderstanding we replace “carriers of the rs11575892 polymorphism” with “carriers of the rs11575892 T allele” in the Abstract. We would like to argue that according to our knowledge it is the first time when minor allele of AGRP polymorphism is associated with increased BMI. Both the articles mentioned by the referee describe association of either major allele of rs5030980 (Ala67) with obesity or homozygotes of minor allele (Thr67) with number of leanness related phenotypes.

The association of a minor allele with body composition phenotypes, here a lower fatness, was reported earlier, as the association with higher values of obesity for a major allele. Both indicated a similar effect of AGRP on body composition. What is the point of the authors on their "new" association? They cannot state that this is an association of the T allele since they did not test yet TT homozygotes. Also, they cannot claim at an association of a minor allele at this point but only at an effect of heterozygotes.

We would also like to point that Referee #3 agrees on the fact that this is the first time when SNP in AGRP is associated with increased BMI.

Not true since the paper of Argyropoulos.

3. Referee’s comment: “In Methods, it makes no sense to use an ANOVA knowing the importance on BMI of covariate effects such as age and gender.”

3. Answer: We agree that age and gender should be obligatory included as covariates when BMI is analysed. However we think that inclusion of uncorrected ANOVA results would give more information to evaluate effects of these covariates on association of SNP with BMI. Besides ANOVA was used also to identify non-genetic factors that are associated with BMI.

This did not give more information but false information. This should be skipped. Significance of co-variates could be tested in the ANCOVA.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests’