Reviewer's report

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

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Reviewer: Inga Prokopenko

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Manuscript from I. Kapa et al. describes the association with increased BMI and a rare variant rs11575892 in the second intron of AGRP gene observed in a sample of 698 elderly Latvians. AGRP gene seems to be an interesting candidate coding for Agouti related protein that interacts with melanocortin receptors, coded by genes known to be associated with monogenic obesity and recently described in large genome-wide meta-analyses for association with increased BMI.

In the sequencing experiment Kapa and colleagues identified only rare variants within the coding and 5’ untranslated regions of AGRP gene. Authors selected only rs11575892 for subsequent genotyping and association testing in the whole sample.

Major Compulsory Revisions

It is not clear, why at least one additional polymorphic variant (among two SNPs in complete LD rs34123523 or rs5030980) wasn’t genotyped in the studied sample. These variants have been previously associated with obesity, but it is not known, whether the association with obesity is observed within the studied Latvian sample. Such genotyping would be important for overall evaluation of the role of AGRP in the increased BMI.

As far as all observed variants within this gene are rare (only 3 heterozygotes were observed in the sequenced sample of 95 subjects), it’s not possible to exclude that in the whole sample rare allele of rs11575892 might be present in the subjects with rare variant of rs34123523 or rs5030980, i.e. some LD might be observed between these three variants.

Authors performed permutation analyses to check whether the association was observed by chance. However, genotyping of the studied polymorphic variant in an additional replication sample would be also desired.

Discretionary Revisions

Power calculation would be helpful to evaluate, whether the sample size of the cohort is adequate to demonstrate such association with sufficient certainty.

A note about how the secondary RNA structure could be affected by other two
SNPs would be useful.

Minor Essential Revisions

Table 2 has a lot of comments in the legend, thus, is quite difficult to read, while the content is quite simple. This table could be divided into 2 parts (having separate 2 rows for each analysis) for ANOVA and GLM analyses. It would be clearer to put two sub-headers for analysis considering BMI as continuous trait and for categorical analyses where sample was divided by median BMI.

Table 1 and Table 3 could be merged in order to make clearer the distribution of associated traits and their loading as covariates in the association with BMI.

Table 1 header “Values” is not clear because the Sub-header “Percentage of patients (n)” is placed in the other column, it would be clearer to put it in the second column.

MC4 and MC3 receptors need to be referenced with their full name.
All gene names should be in Italic characters.