Author's response to reviews

Title: A study of the relationships between KLF2 polymorphisms and body weight control in a French population

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

Please find enclosed the revised manuscript entitled ‘A study of the relationships between KLF2 polymorphisms and body weight control in a French population’ we would like to publish in BMC Medical Genetics as a research article.

We answered properly all the points raised by the referee and we do hope the manuscript is now ready to be published in BMC Medical Genetics. We believe our data are interesting to those with obesity related research interests.

Kind regards,

Aline Meirhaeghe
We thank the referee for his helpful comments. We answered each point as requested.

**Major compulsory revisions:**

1. The regions of the gene and the number and the phenotype of the subjects (obese/lean) that were sequenced were described in the result section: “Exons 1, 2 and 3 as well as the totality of intron 1 and the last 183 bp of intron 2 were sequenced in 10 (5 obese and 5 non-obese) subjects.” We guess the referee did not see this paragraph. We do not have power to detect rare variants (frequency less than 10%) but in our case, common and not rare variants are needed to perform association studies with enough statistical power.

2. Restriction digests are routinely used in our lab. First, we avoid incomplete digestions by performing the digestion reaction overnight. Second, we try, when possible, to have a SNP-independent internal restriction site in the PCR fragment. This was, for example, the case for the KLF2 3’UTR 1239C>A polymorphism. When there is no such restriction site, we add the restriction enzyme in excess. Moreover, around 20% of the “uncut” PCRs are controlled by digesting them again.

3. It is true variants in the 5’UTR of the genes are essential to look at. We tried but did not manage to amplify and sequence properly the proximal promoter of the KLF2 gene despite the design of several pairs of primers. As by the time we finished the sequencing of the gene, some SNPs (including all the ones we had detected ourselves) in KLF2 were listed in the NCBI dbSNP database, we noted that one SNP was in the 5’UTR of the gene (rs8106384). We genotyped 59 individuals for this SNP but found only 1 C/T heterozygote (T allele frequency below 1%). Therefore, we did not type this SNP.

   We agree that more than 2 SNPs should be typed before to conclude definitely about the role of the KLF2 gene on body weight control but we have been careful to limit our conclusions to the two SNPs we studied and not to the KLF2 gene itself.

**Minor essential revisions:**

1. We replaced LKLF by KLF2 as requested.

2. It is true the $r^2$ gives a better information than the D’. We added the $r^2$ value (0.036) in the text page 7.

3. We added in the legend of table 5 the significance of the p value :”p : p value for global haplotype effect.”
Regarding the SEM algorithm, it has been developed by D.A. Tregouet and colleagues and since, has generated between 10 and 20 publications by different teams (including ours). I am not an expert in those mathematical models but the referee can read the reference (Tregouet et al. *Ann Hum Genet* 2004) that compares the two algorithms.

4. Regarding the power calculation, we used the numbers of subjects in each genotype group (depending on the frequency of the KLF2 polymorphisms) using a dominant model i.e. two genotype groups, the means and standard deviations of BMI, by gender, as displayed in tables 3 and 4. We better explained this in the method section page 6: “Power calculations were based on a two-sided, two-sample (dominant model), t-test, with a power of 0.80, an $\alpha$ of 0.05, the numbers of subjects in each genotype group and the means and standard deviations of BMI displayed in tables 3 and 4, in men and women respectively.” And in the discussion page 8: “The population-study we used is powerful enough (power $\geq$80%, $\alpha$=5%) to detect a difference in BMI of 1.0 and 1.4 kg/m² in men and women respectively for both polymorphisms.”

We did not type the intron 1 variant as its frequency was quite low (below 10%) (weak power for the statistical analyses) and was not located in a known transcription factor binding site (assessed by the MatInspector software) and therefore unlikely to have a functional impact on the gene expression. We added the frequency of the intron 1 variant in the manuscript to better explain why we did not type this one. Also we wrote in the result section page 7: “We therefore decided to genotype the Lille population study for the Pro104Leu and 3‘UTR 1239C>A polymorphisms as they were frequent enough to give a sufficient statistical power and more likely to have a functional impact at the protein or gene expression level.”.