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.

Major compulsory revisions:

1. Concerning the PCR-RFLP method, performing blinded duplicates is one solution to avoid genotyping errors when there is no internal site that is cut. An alternative solution is to add the enzyme in excess to avoid partial cutting and to repeat the digestion for the “uncut” PCR products. In our lab, we add the enzyme in excess for all the digestion reactions and we check 20% of the “uncut” PCR products by digesting them again. If the percentage of “uncut” products becoming “cut” after this verification is low, then we assume the genotyping error rate is low. If this percentage is high (above 5%), then all the “uncut” PCR products are digested again. Fortunately, this case is extremely rare in our hands. Also, a high genotyping error rate often leads to a sample for whose the Hardy-Weinberg equilibrium is not respected. That was not the case for the KLF2 polymorphisms tested. Therefore, we can assume the genotyping error rate in this study is very low.

2. We now added in the result section of the manuscript (page 7) that we could not sequence the 5’ UTR of KLF2: “We were unable to amplify and sequence properly the proximal promoter of KLF2 (up to 400 bp upstream the ATG) despite attempts with several pairs of primers. However, one SNP (rs8106384, -18T>C) in the 5’ UTR (18 bp before the ATG) was displayed on the NCBI dbSNP web site. We checked the existence of this polymorphism but found only one heterozygote in 59 individuals (-18T allele frequency < 1%).”

3. We simplified table 2 and added that the p values were indeed for the 3*3 analyses. We also changed the format of tables 2-4 (portrait).

4. We added a paragraph in the discussion addressing the limitations of this study:
   “Our study has both strengths and weaknesses. It was conducted in a large random sample of population which avoids possible bias due to recruitment in hospitals or clinics and university staff or students. The analyses were performed in men and women separately which is an important advantage with regard to the impact of gender on fat mass depots and obesity risk. A difference in BMI between genotype groups should be at least of 1 kg/m² to be considered phenotypically relevant. The population-study we used was powerful enough (power ≥ 80%, α=5%) to detect a difference in BMI of 1.0 and 1.4 kg/m² in men and women respectively for
both polymorphisms. The major limitation of the study may be that only two SNPs in \textit{LKLF} were studied and therefore we can not totally exclude that other SNPs in or nearby (especially in regulatory regions) the KLF2 gene might be associated with obesity traits.

**Minor essential revision :**

1. The manuscript has been corrected by an English native speaker.