Author's response to reviews

Title: Risk Interaction of Obesity, Insulin Resistance and Hormone-Sensitive Lipase Promoter Polymorphisms in the Development of Fatty Liver

Authors:

Pi-Jung Hsiao (pihsiao@cc.kmu.edu.tw)
Zhih-Cherg Chen (z_cchen@yahoo.com.tw)
Wei-Wen Hung (hung4488@ms57.hinet.tw)
Mei-Yueh Lee (lovellelee@hotmail.com)
Jee-Fu Huang (49581110@cc.kmu.edu.tw)
Kung-Kai Kuo (kuoksfo@yahoo.com.tw)

Version: 3 Date: 5 March 2013

Author's response to reviews: see over
Dear Editor-in-Chief, *BMC Medical Genetics*:

We are very grateful for the expert comments from the three reviewers, which were very helpful for the revision of our manuscript. We have addressed the questions and suggestions raised by the reviewers as follows:

**Response to Reviewer 1: Yuzuru Otsuka**

1. The official name of HSL is lipase, hormone-sensitive and official symbol is LIPE, so better to change to official name and symbol.

   **Ans:** We agree and we have replaced HSL with the official name and symbol. The title has been changed to “Risk Interaction of Obesity, Insulin Resistance and **Hormone-Sensitive Lipase Promoter Polymorphisms (LIPE-60 C > G)** in the Development of Fatty Liver”. In the abstract, line 1 now reads ”Hormone sensitive lipase (HSL) promoter (LIPE-60 C > G) polymorphism” and **Methods** paragraph 3, line 4 now reads “HSL promoter polymorphism (rs34845087 for C-60G, **LIPE 14672 C>G**)”.

2. The data in the paper is limited on the association between genotype and clinical data, therefore, the Anova or t-test results grouped by genotype or (GG+GC) and **CC** (further grouped by GI or not) will show the effect of the genotype.

   **Ans:** This study was designed to analyze the risk impact and interaction of confounding factors in the development of fatty liver and related metabolic abnormalities. Therefore, we only reported the results from the multivariate regression analysis based on the expert recommendations of our Statistical Analysis Laboratory. Department of Medical Research. It demonstrated no significant effect on fatty liver.
formation but a significant contribution to raised serum triglyceride levels by
genotype (CG+GG vs CC) as reported in table 2 and 4. To make this easier to
understand, we revised the 5th paragraph, last 4 lines, in the discussion to say, “We
observed no difference in anthropometric or metabolic parameters and related
insulin resistance indexes between genotype (CG + GG) and (CC) carriers in the
NTG group, except for significantly higher serum TG levels found in carriers of
the G allele in the GI group (supplementary tables).”

3. There are several papers describe the association of this polymorphism and
triglyceride etc, but your paper did not describe the previous papers. For
example, Talmud et al. (2005, Nutr Metab Card Dis 15, 31-35) and Talmnd et al.
(2002, Nutr Metab Card Dis 12 173-177) showed no relation between TG and
genotype. But Pilajamaki et al. (2001 Eur J Clin Invest 31, 302-308) and Sone et
al. (2010, J Nutr Sci Vitaminol (Tokyo) 56, 123-131) showed the minor allele
decreased TG in blood. Furthermore, knock out mice of LIPE decreased TG in
blood and liver (Haemmerle 2002, JBC 277, 12946-12952). From those previous
papers your result in GI group is different. So please discuss the genotype effect
on clinical data in detail.

Ans: We sincerely appreciate the references to these papers. As the reviewer mentions,
the previous reports had inconsistent results and no consecutive link between
genotypes and phenotype. We have reviewed these related articles and cited them as
references 18, 32, 33, and 34. One paragraph (5th paragraph) was added to the
discussion as suggested. The discrepancy between our results and previous reports
may stem from the following: 1. Our study population is substantially much larger
than the previous reports. 2. Glucose can affect serum triglyceride because
hyperglycemia can activate ShREBP, which additionally transcriptionally activates
lipogenic genes. Thus the glucose level can be regarded as a confounding factor, and
needs to be standardized by stratification.
We used a quantitative linear regression method to measure the “impact weight” on TG in the NGT and GI groups for the metabolic risk factors (Table 4). 3. The LIPE knock-out mouse model used in some earlier studies is relatively difficult to apply to human studies. Risk interactions in human studies include the effects of lifestyle, diet, race and gender and it is hard to analyze these interactions in a mouse model.

**Response to Reviewer 2: Dominique Langin**

1. The power of the study and the lack of replication are of concern. For analyses of surrogate parameters of insulin resistance such as HOMA-IR and other clinical parameters, the number of individuals is limited compared to published studies. The impact of the hormone-sensitive lipase promoter -60 C>G polymorphism on triglyceride levels in the glucose intolerant group (n=299) seems marginal especially as the glucose intolerance group is a heterogeneous mix of conditions as it encompasses all individuals with fasting glucose > 100mg/dl. Classification according to the presence and absence of the metabolic syndrome was of interest in the context of the study. However, waist circumference is not reported.

   **Ans:** We appreciate the importance of these comments. In terms of single nucleotide polymorphism and insulin index (HOMA-IR), our study population was relatively larger and more standardized compared with previous reports which only included hundreds of subjects.

   Our sample size is sufficient for statistical analysis to evaluate the risk interaction by multivariate analysis, as validated by our statistical expert. As the reviewer stated, glucose homeostasis significantly contributes to fatty liver formation and serum triglyceride. That was why we excluded overt diabetes, lipid-lowering drug users and any medical conditions which would confound the analysis during our detailed medical review process. The serum triglyceride level is dominantly affected by (1) VLDL-C, which is secreted by liver and regulated by insulin, (2) hepatic de novo lipogenesis and (3) serum glucose level. Therefore, the serum glucose level should be
stratified for normal glucose level and glucose intolerance to reduce the interference for analysis.

We stratified the whole population into “normal glucose tolerance (NGT, <100mg/dl)” and “glucose intolerance (GI, ≥100mg/dl)” groups. The latter group included impaired fasting glucose (IFG, n= 229) and newly-diagnosed DM (n=70). If insulin resistance is highly concerned, the latter group is not heterogeneous.

Table 2 of our supplemental file shows that the TG level was significantly higher in the CG+GG genotype than in the CC group (176.5 vs 142.6 mg/dl; p=0.023, nonparametric Mann-Whitney rank-sum test). To clarify the interaction of metabolic risk factors, we used multiple regression analysis. The results showed that the HSL promoter genotype had a very significant impact on the TG level. (p=0.010, Table 4) Compared to the CC genotype, the CG+GG genotype can increase the TG level by an estimated impact of 34.10, which is higher than any other index, and this effect is not marginal (Table 4) but statistically significant.

Because Men’s BMI is reported to predict approximately 93% of the variance linked to obesity-related adipocyte size, fat cell mass and fat distribution, the waist circumference was not used for defining obesity.

2. Interpretation of the impact of the hormone-sensitive lipase promoter -60 C>G polymorphism focuses on the action of the enzyme in the liver. However, the lipase is expressed at much higher levels in adipose tissue where it controls the release of fatty acids into the bloodstream and hence liver exposure to fatty acids. Therefore, it is likely that the impact on lipid metabolism of variations in hormone-sensitive lipase activity will be influenced by its level of expression in adipose tissue.

**Ans:** We absolutely agree that the serum free fatty acid mainly comes from adipose lipolysis which is determined by the HSL activity. The serum triglyceride level is dominantly affected by (1) VLDL-C, which is secreted by liver and regulated by
insulin, (2) hepatic de novo lipogenesis and (3) serum glucose level. Therefore, the serum glucose level should be stratified by normal glucose level and glucose intolerance to reduce the confounding interference for analysis.

Table 2 of our supplemental file shows there was no significant difference in serum non-esterified fatty acid levels (NEFA) between the CC and CG+GG genotypes in the normal glucose tolerance and glucose intolerance groups. This means the genetic effect of HSL (C-60G) plays no significant role in regulating the fasting lipolysis from adipocytes. The anti-lipolytic effect of insulin for adipose tissue is the most sensitive compared to muscle and liver. (Peter Kovas, 2005, Best Pract Res Clin Endoc Metabol; vol 19:625-35) The GI group in our study recruited mainly pre-diabetes or new-onset DM patients, at a relatively earlier stage of insulin resistance. Thus the anti-lipolytic effect of insulin is quite enough to overcome the genetic effect of HSL. Insulin regulates the hepatic glucose output for fasting glucose and VLDL-C secretion for serum TG. Our data revealed a significant difference in serum TG between the NGT and GI groups (Table 4). This is probably due to differences in insulin sensitivity and the genetic effect of HSL(C-60G) in liver and adipose tissue. Our point of view is coherent with the other studies, as discussed now in the last paragraph of discussion section, line 1-11. We believe the impact on lipid metabolism of variations in HSL activity is not related to the NFFA released from adipose tissue into the bloodstream.

**Response to Reviewer 3: Johan Jocken**

This is an interesting study however a major concern is that stratification of the population is carried out based solely on fasting glucose levels. An oral glucose tolerance test or clamp would have been a more appropriate technique to define a glucose (in)tolerance state. Therefore, I suggest reanalysing the data without stratification.
We completely agree that an OGTT or glucose clamp would be the gold standard for evaluating glucose tolerance. However, budgetary limitations made this clinically impractical. To overcome this limitation, we stratified the glucose effect first. Insulin resistance was analyzed and compared with HOMA-IR and Adipo-IR, which are well known indexes for measuring insulin resistance in research and clinical applications. As a matter of fact, we initially analyzed our data without glucose stratification. The unstratified results showed no significant correlation between HSL genotypes and any serum metabolic parameters. After glucose stratification, there were statistically significant differences in serum TG among different HSL genotypes. (Supplemental Table 2) These results suggest that the genetic effect of HSL (C-60G) polymorphisms is truly influenced by insulin resistance.

Finally, we have had a native English writer redact the revised manuscript for publication.