June 1, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3173-review.doc).

Title: Association of UCP3 gene polymorphisms with nonalcoholic fatty liver disease in Chinese children

Author: **Yan-ping Xu, Li Liang, Chun-lin Wang, Jun-fen Fu, Pei-ning Liu, Lan-qiu Lv, Yi-min Zhu**

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3173

Thank you for the review of our manuscript, Manuscript revision NO: 3173, for consideration for publication in *World Journal of Gastroenterology*. As you suggested, we have carefully considered the comments of all two reviewers and accordingly implemented their suggestions as detailed below:

1. Reviewer1: This is an interesting manuscript about NAFLD in children and UCP3 polymorphisms. The manuscript has adequate methodology and is good written. However, some improvment is need.

1) In the abstract the authors need add NAFLD in children and healthy children (control).

METHODS: A total of 250 NAFLD (147 males and 103 females) and 200 healthy individuals (control, 109 males and 91 females), aged between 6 and 16 years were enrolled in this study. The four nonsynonymous SNPs in the UCP3 gene: polymorphisms of rs1726745, rs3781907, rs11235972 and rs1800849 were genotyped using MassArray. Body mass index (BMI), waist and hip circumference, blood pressure (BP), fasting blood glucose (FBG), insulin, lipid profiles were

measured and performed B-ultrasound examination in all the subjects.

2) In the methods again subjects helthy children (control).

Blood samples (n = 200) were also taken from healthy examination individuals as control (109 males and 91 females) in 2011, all of who were from department of Child Health Care, The Affiliated Yuying Children's Hospital of Wenzhou Medical University and Ningbo Women & Children's Hospital. The protocol was approved by the Medical Ethics Committee of The Children's Hospital of Zhejiang University School of Medicine. Written informed consent from parents (or guardians) and children (where appropriate) were obtained.

3)I think that discussion must be reformulated because is confuse and do not have some references children studies of NAFLD and metabolic syndrome. Also, the authors must be include one paragraph talking about the limitation of IMC in children, because the best it was evaluate with percentil.

We have revised to see detail in manuscript.

4) The english needs improve.

We have revised to see detail in manuscript.

2. Reviewer2:

1) Authors should explain how they chose control groups. Were they hospital based patients, or community based persons. Are there any reason to select fewer number of control than cases.

Blood samples (n = 200) were also taken from healthy examination individuals as control (109

males and 91 females) in 2011, all of who were from department of Child Health Care, The Affiliated Yuying Children's Hospital of Wenzhou Medical University and Ningbo Women & Children's Hospital. The protocol was approved by the Medical Ethics Committee of The Children's Hospital of Zhejiang University School of Medicine. Written informed consent from parents (or guardians) and children (where appropriate) were obtained. We chose control group aged between 6 and 16 years and blood samples were limited.

2) The confirmation method of NAFLD should describe better.

NAFLD children (147 males and 103 females) were referred to our endocrinology department from January 2006 to September 2011; NAFLD was defined according to the revised definition and treatment guidelines for NAFLD by the Chinese Hepatology Association in February 2006, and was diagnosed by means of a protocol using clinical, laboratory and ultrasound examinations in combination. In this study, NAFLD was diagnosed as a diffusely echogenic change on liver B-ultrasonography (fatty infiltration in liver), with or without elevated serum aminotransferase levels and other factors that can cause liver fatty infiltration or aminotransferase elevation, such as infectious hepatitis (hepatitis B and C, Epstein-Barr virus infection), drug-induced hepatitis, and some metabolic diseases were excluded. None of the subjects had history of alcohol consumption.

3) Multivariete analysis prosedures might define well.

Multivariate logistic regression analysis by using stepwise selection was constructed to determine which of the potential risk factors of NAFLD were. Given the BMI, HOMA-IR, ALT, TCHO, rs1726745, rs3781907, rs11235972, and rs1800849 risk factors relative to the potential number of variables in our model, only those variables that had the highest possibility for

independent prediction of outcome in our logistic regression were included. Multivariate logistic regression analysis was performed to estimate the odds ratios (ORs) and 95%confidence intervals (CI) for the potential risk factors of NAFLD.

4) Tables have some weakness. Forexample it's confused in Table 2 different genotype options. The homozygosity and heteroygosity situations not clear. Additionally tables need more detailed legends. Abbrivations should describe there.

As suggested, tables' format has been updated.

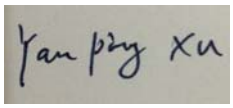
5) Why just rs1800849 included multivariete analysis. It's unsignificant as others.

We have revised to see detail in manuscript and table3.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



Yanping Xu Ph.D

The Children's Hospital of Zhejiang University School of Medicine

57 Zhugan Xiang, Hangzhou 310003, China.

Tel: +86--571-87061007

Fax: +86-571-87033296

Email: xuyanping726@hotmail.com