Letter to the Editor

Screening for Melanocortin 4 Receptor Mutations in Chinese Extremely Obese Individuals*

YANG Jian Jun[1,&], TANG Shan Shan[2,3,4,5,&], HU Cheng[2,3,4,5], ZHANG Rong[2,3,4,5], SONG Zhi Cheng[1], WANG Bing[1], YU Wei Hui[2,3,4,5], LU Jing Yi[2,3,4,5], JIANG Feng[2,3,4,5], BAO Yu Qian[2,3,4,5], GU Yan[1,#], and JIA Wei Ping[2,3,4,5,#]

Accumulating evidence indicates that overweight and obesity are the major international public health concern[1]. Obesity is a major independent risk factor for chronic diseases, such as hypertension, type 2 diabetes, cardiovascular disease, stroke, and certain cancer. Disease burden due to obesity has been dramatically increasing in many countries including China in the past years. According to the Nationwide Health and Nutrition Survey (CHNS), the prevalence of overweight and obesity among men and women in China increased by 17.6% and 8.8%, respectively, from 1993 to 2009[2].

Genetic predisposition plays an important role in obesity. Polygenic obesity and monogenic obesity are related with single gene mutation such as *proopiomelanocortin (POMC), leptin receptor (LEPR), leptin (LEP), proconvertase 1 (PC1), melanocortin 4 receptor (MC4R), and melanocortin 3 receptor (MC3R)*. Of them, *MC4R* mutation is most common and causes monogenic human obesity. The *MC4R*-encoded protein belongs to the melanocortin receptor family that is composed of 5 genes (*MC1R* to *MC5R*). Human *MC4R* located on chromosome 18q22 is a 332 amino-acid and G-protein coupled receptor and mainly expressed in hypothalamus para-ventricular nucleus[3]. Owing to its special location anatomically, *MC4R* plays an important role in regulating food intake and energy homeostasis when it is activated by endogenous α-melanocyte stimulating hormone (α-MSH) produced by pro-opiomelanocortin (POMC) and antagonized by agouti-related protein (AGRP).

It has been shown that frequent variants in and near *MC4R* are closely related to certain traits of human obesity, such as BMI, fat mass, and food intake. Frameshift mutation in *MC4R* was first reported in 1998 and the crucial role of *MC4R* in early-onset of obesity was then established[4]. At present, more than 150 *MC4R* mutations have been identified in human obesity of different ethnic origin including frameshift, inframe deletion, nonsense and missense mutations that are closely related to early-onset and/or morbid obesity[5]. Since few studies are avaible on *MC4R* mutation in China, *MC4R* mutations in 35 severely obese subjects from Shanghai were investigated and the relation between its clinical phenotype and genotype was analyzed in this study.

Thirty-five Chinese Han obese participants with their BMI≥32 kg/m^2 and 100 Chinese Han control subjects with their BMI<25 kg/m^2 in Shanghai were enrolled in this study. The study was approved by the Institution Review Board of Affiliated Ninth People's Hospital of Shanghai Jiao Tong University according to Helsinki Declaration II. Written informed consent was obtained from each participant. The clinical characteristics of the subjects are listed in Table 1.

Table 1. Clinical Characteristics of Subjects Included in this Study

	Cases	Controls
Male/female (*n*)	16/19	47/53
Age (years)	24 (19,33)	31 (23,35)
BMI (kg/m^2)	40.64±7.70	21.58±1.94

Note. BMI: body mass index. Data are expressed as mean±SD.

All participants underwent a detailed clinical investigation. Anthropometric parameters were measured, such as body height, body weight, blood pressure, body mass index (BMI), waist and hip circumstance. Severe obese subjects without history of diabetes underwent OGTT and were assessed using standard 75 g overnight fasting glucose. Blood samples were taken for DNA analysis and

doi: 10.3967/0895-3988.2013.07.014

1. Department of General Surgery, Affiliated Shanghai Ninth People's Hospital of Shanghai Jiao Tong University Medical School, Shanghai 200011, China; 2. Department of Endocrinology and Metabolism, Affiliated Sixth People's Hospital of Shanghai Jiao Tong University, Shanghai 200011, China; 3. Shanghai Diabetes Institute, Shanghai 200011, China; 4. Shanghai Key Laboratory of Diabetes Mellitus, Shanghai 200011, China; 5. Shanghai Clinical Center for Diabetes, Shanghai 200011, China

biochemistry, such as serum glucose, insulin, TC, TG, FFT_3, FFT_4, TSH, and ACTH.

Genomic DNA was extracted from peripheral blood leucocytes in the whole-blood samples. The coding region of *MC4R* was divided into 6 fragments and amplified by PCR using the designed pairs of primers as previously described[6] in a thermal cycler (Veriti, ABI, USA). The amplified PCR fragments of *MC4R* were detected by polyacrylamide gel electrophoresis and sequenced directly using the 3100 genetic analyzer (Applied Biosystems, USA).

The functional consequence of nonsynonymous mutation was evaluated with PolyPhen2 (http://genetics. bwh.harvard.edu/pph2) which can predict the impact of amino acid substitution on the function and structure of human protein.

The whole coding sequence of *MC4R* was screened. Two mutations (missense heterozygous mutation G231S and nonsense mutation C277X) associated with severe obesity in Chinese individuals were observed (Figure 1) but no in control group. The nonsense mutation C277X was also identified in Chinese children and adolescents in another study[7].

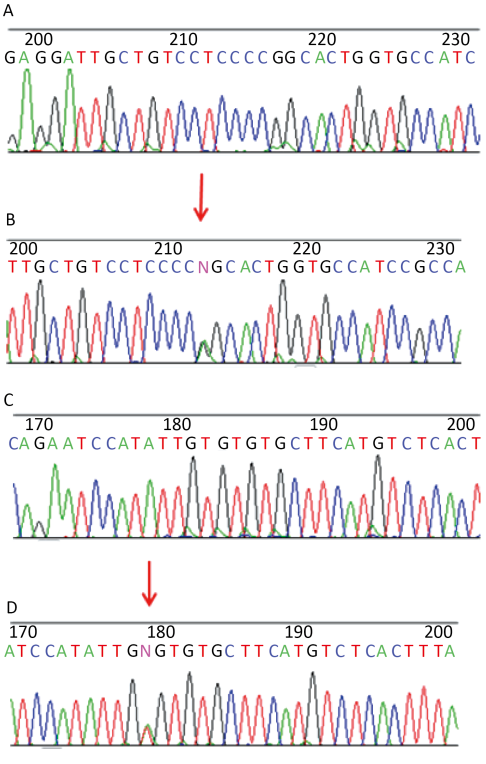

Figure 1. Normal sequence (A), heterozygous G231S mutation sequence (B), normal sequence (C), and nonsense mutation C277X sequence (D) of *MC4R* mutation.

The nonsynonymous mutation led to serine substitute for glycine at the amino acid residue 231 of its receptor. PolyPhen2 showed that the mutation was possibly damaged with a score of 0.940. The carrier was a woman (age: 33 years, height: 162 cm, weight: 141.61 kg, BMI: 53.96 kg/m^2). Beside obesity, she also had diabetes, metabolic syndrome, hypertension, severe fatty liver disease, hyperthyreosis and sleep apnea syndrome with a high FT_3 and FT_4 level (Table 2). The nonsense mutation was found in a 16-year-old obese boy with a BMI of 35.24 kg/m^2 (height: 183 cm, weight: 118 kg). His weight increased uniformly from childhood. He also exhibited severe insulin resistance (fasting insulin=82.18 μl/mL, HOMA-insulin resistance index =17.17) with a high TC level (2.62 mmol/L). The other biochemical indexes including ACTH, TSH, FT_3, FT_4 were in the normal range.

It was reported that most *MC4R* mutations are associated with severe obesity and lead to intracellular retention of its receptor[8]. C277X belongs to

Table 2. Clinical Features and Hormone Level in 2 Mutation Carriers

	G231S	C277X
Clinical data		
Gender	female	male
Age (years)	33	16
Height (cm)	162	183
Weight (kg)	141.60	118.00
BMI (kg/m^2)	53.96	35.24
Metabolic hormones levels		
FPG (mmol/L)	ND	4.70
FIN (2.60-24.90 ul/mL)	ND	82.18
HbA1c (4.7%-6.4%)	6.5	5.50
TC (0.56-1.70 mmol/L)	ND	2.62
TG (2.33-5.70 mmol/L)	ND	3.94
HDL (0.80-1.80 mmol/L)	ND	0.82
LDL (1.30-4.30 mmol/L)	ND	2.22
FT3 (1.71-3.71 pg/mL)	6.51	4.15
FT4 (0.70-1.48 ng/dL)	3.40	1.04
TSH (0.34-5.60 uIU/mL)	0.03	2.35
ACTH (12-78 pg/mL)	ND	58.10

Note. BMI: body mass index; FPG: fasting plasma glucose; FIN: fasting insulin; TC: total cholesterol; TG: triglyceride; FFT_3: free T_3; FFT_4: free T_4; TSH: thyroid-stimulating hormone; ACTH: adrenocorticotropic hormone; ND: not determined. Abnormal levels are highlighted in bold.

class I and can induce complete functional loss of its receptor and defective protein synthesis[9]. G231S is significantly different from C277X., its cell surface expression level remains normal[10], its basal activity (also known as constitutive activity) is essential for long-term energy homeostasis, and PolyPhen2 shows that the mutation is possibly damaged, indicating that G231S may be pathogenic.

The present study has the following limitations. First, the sample size was relatively small and the variant frequency was therefore not calculated, which might be the reason why the reported mutations of *MC4R* were not detected in China. Beside, since pedigrees and phenotypes were not available from the two mutation carriers, whether the mutations were co-segregated with obesity individuals in China remains unknown. More clinical data and personal information, such as history of overweight, body weight, waistline, visceral and subcutaneous fat are thus needed to draw a definite conclusion.

In the present study, two mutations in *MC4R* associated with severe obesity in Chinese individuals were identified. Further large sample screening and functional investigation are needed to confirm our findings and elucidate the mechanism underlying such association between the variants and obesity.

ACKNOWLEDGEMENTS

The authors highly appreciate all the participants in this study and all the colleagues at Department of General Surgery, Shanghai Ninth People's Hospital and Shanghai Clinical Center for Diabetes for their cooperation and support.

*This work was supported by Shanghai Rising-Star Program (12QH1401700), the Drug Innovation Program of the National Science and Technology Project (2011ZX09307-001-02), Science Foundation of Shanghai Municipal Health Bureau (2012291). Excellent Young Medical Experts of Shanghai (XYQ2011041), Program of Shanghai Municipality for Basic Research (11JC1409600).

#Correspondence should be addressed to JIA Wei Ping and GU Yan. Tel: 86-21-2405892, Fax: 86-21-64368031, E-mail: wpjia@sjtu.edu.cn; Tel: 86-21-23271699, Fax: 86-21-63136856, E-mail: yangu@sjtu.edu.cn

&YANG Tian Jun and TANG Shan Shan contributed equally to this work.

Biographical notes of the first authors: YANG Jian Jun, male, born in 1977, M.D., majoring in general surgery. TANG Shan Shan, female, born in 1987, PH.D., majoring in endocrinology and metabolism.

Received: January 9, 2013;
Accepted: May 28, 2013

REFERENCES

1. Lau DC, Douketis JD, Morrison KM, et al. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. CMAJ, 2007; 176, S1-S13.
2. Xi B, Liang Y, He T, et al. Secular trends in the prevalence of general and abdominal obesity among Chinese adults, 1993-2009. Obes Rev, 2012; 13, 287-96.
3. Gantz I, Miwa H, Konda Y, et al. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J Biol Chem, 1993; 268, 15174-9.
4. Vaisse C, Clement K, Guy-Grand B, et al. A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet, 1998; 20, 113-4.
5. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocr Rev, 2010; 31, 506-43.
6. Gu W, Tu Z, Kleyn PW, et al. Identification and functional analysis of novel human melanocortin-4 receptor variants. Diabetes, 1999; 48, 635-9.
7. Wang CL, Liang L, Wang HJ, et al. Several mutations in the melanocortin 4 receptor gene are associated with obesity in Chinese children and adolescents. J Endocrinol Invest, 2006; 29, 894-8.
8. Lubrano-Berthelier C, Durand E, Dubern B, et al. Intracellular retention is a common characteristic of childhood obesity-associated MC4R mutations. Hum Mol Genet, 2003; 12, 145-53.
9. Tao YX. Molecular mechanisms of the neural melanocortin receptor dysfunction in severe early onset obesity. Mol Cell Endocrinol, 2005; 239, 1-14.
10. Lubrano-Berthelier C, Dubern B, Lacorte JM, et al. Melanocortin 4 receptor mutations in a large cohort of severely obese adults: prevalence, functional classification, genotype-phenotype relationship, and lack of association with binge eating. J Clin Endocrinol Metab, 2006; 91, 1811-8.