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BRIEF ARTICLE

Association between *UCP3* gene polymorphisms and nonalcoholic fatty liver disease in Chinese children

Yan-Ping Xu, Li Liang, Chun-Lin Wang, Jun-Fen Fu, Pei-Ning Liu, Lan-Qiu Lv, Yi-Min Zhu

Yan-Ping Xu, Jun-Fen Fu, Department of Endocrinology, Children's Hospital of Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China
Li Liang, Chun-Lin Wang, Department of Pediatrics, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China
Pei-Ning Liu, Department of Child Health Care, the Affiliated Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China
Lan-Qiu Lv, Department of Child Health Care, Ningbo Women and Children's Hospital, Ningbo 315000, Zhejiang Province, China
Yi-Min Zhu, Department of Epidemiology and Biostatistics, Key Laboratory of Reproductive Genetics, Ministry of Education, Zhejiang University, Hangzhou 310003, Zhejiang Province, China
Author contributions: Xu YP performed the research, statistical analysis and wrote the manuscript; Liang L contributed to the conception and design of the study; Wang CL and Fu JF contributed to collecting and processing the data; Liu PN, Lv LQ and Zhu YM contributed to the control group blood collecting.
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Correspondence to: Li Liang, Professor, Department of Pediatrics, the First Affiliated Hospital, College of Medicine, Zhejiang University; 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. zdliangli@163.com
Telephone: +86-571-87061007 Fax: +86-571-87033296
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Abstract

AIM: To confirm the hypothesis that polymorphisms of the uncoupling protein 3 (*UCP3*) gene are associated with the occurrence of nonalcoholic fatty liver disease (NAFLD).

METHODS: A total of 250 NAFLD patients (147 males and 103 females) and 200 healthy individuals who served as controls (control, 109 males and 91 females), aged between 6 and 16 years were enrolled in this study. The four non-synonymous single nucleotide polymorphisms (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 and lipid profiles were measured and B-ultrasound examination was performed in all subjects.

RESULTS: NAFLD patients showed risk factors for metabolic syndrome: elevated BMI, waist-to-hip ratio, BP, FBG, homeostasis model assessment-estimated insulin resistance, total triglyceride, total cholesterol and low-density lipoprotein-cholesterol, while decreased high-density lipoprotein-cholesterol level compared with the control group. The GG genotype distributions of rs11235972 in the NAFLD group differed significantly from that in the control group. We found that waist circumference between CC (58.76 ± 6.45 cm) and CT+TT (57.00 ± 5.59 cm), and hip circumference between CC (71.28 ± 7.84 cm) and CT+TT genotypes (69.06 ± 7.75 cm) were significantly different with and without rs1800849 variation ($P < 0.05$).

CONCLUSION: A higher prevalence of rs11235972 GG genotype was observed in the NAFLD group compared with the control group. No differences were observed for the other SNPs. However, there was a significant difference in body height in addition to waist and hip circumference between the CC (mutant type group) and CT+TT group with and without rs1800849 variation.

Key words: Nonalcoholic fatty liver disease; Uncoupling protein 3; Single nucleotide polymorphisms

Core tip: There are few population-based prevalence

studies of pediatric nonalcoholic fatty liver disease (NAFLD). Uncoupling protein 3 (*UCP3*) is considered to be associated with obesity, given the role for *UCP3* in the regulation of energy and lipid metabolism. This is the first study to report that there significant difference of body height, waist and hip circumference between CC (mutant type group) and CT+TT group with and without rs1800849 variation were found. This study confirmed the hypothesis that polymorphisms of the *UCP3* are associated with the occurrence of NAFLD. These variations could be useful for the diagnosis and/or prognosis of NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic condition characterized by abnormal lipid deposition in hepatocytes in the absence of excess alcohol intake. NAFLD comprises a wide spectrum of liver damage, including simple steatosis, steatohepatitis, fibrosis or even cirrhosis of the liver[1]. NAFLD does not only impact adults, but is also one of the major causes of liver diseases in children[2]. There are few population-based prevalence studies of pediatric NAFLD. Some studies have suggested a prevalence of 2.6%-9.6% for suspected NAFLD among children and adolescents in the United States[3,4] and Asia[5,6]. NAFLD has been shown to be associated with metabolic syndrome (MetS), which comprises obesity, type 2 diabetes, dyslipidemia and high blood pressure (BP) with insulin resistance being the central mechanism[7,8]. Theoretically, many variations in candidate genes related to MetS may contribute to the pathogenesis of NAFLD, such as genes related to insulin resistance and genes influencing hepatic free fatty acid metabolism. Elucidation of genetic factors that predispose an individual to NAFLD may lead to the development of non-invasive biomarkers for the early diagnosis of NAFLD and may allow early preventive and therapeutic strategies for those at the high risk.

Uncoupling protein 3 (*UCP3*) gene is located on chromosome 11q13. *UCP3* is a mitochondrial anion carrier protein with a highly selective expression in skeletal muscle, a major site of thermogenesis in humans, which makes an attractive target for studies into the regulation of body weight. Reduced expression of *UCP3* decreases energy expenditure and increased expression of *UCP3* mRNA in muscle is related to an increase in the metabolic rate and to a lower body mass index (BMI)[9,10]. Therefore, *UCP3* may be involved in obesity, given the role of *UCP3* in the regulation of energy and lipid metabolism. Genetic variants of *UCP3* have been identified, and specifically polymorphisms of 55C/T may impact type 2 diabetes mellitus (T2DM), obesity and weight gain[11-13]. This study confirmed the hypothesis that polymorphisms of the *UCP3* are associated with the occurrence of NAFLD. These variations could be useful for the diagnosis and/or prognosis of NAFLD, although the functional significance of *UCP3* polymorphisms is not clear.

MATERIALS AND METHODS

Subjects

A total of 250 NAFLD children and 200 healthy individuals (controls), aged between 6 and 16 years were enrolled in this study. 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[14,15], 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 which 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 a history of alcohol consumption. Blood samples (n = 200) were also obtained from healthy individuals, who served as controls (109 males and 91 females) in 2011 from the Department of Child Health Care, The Affiliated Yuying Children's Hospital of Wenzhou Medical University and Ningbo Women and 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 were obtained from parents (or guardians) and children (where appropriate).

Laboratory assessment

The weight and height of the subjects were measured with a calibrated scale after removing shoes and heavy clothing, if any. BMI is calculated by taking the ratio of weight in kilogram and the square of height in meter. Waist was measured at the midpoint between the lower border of the rib cage and the iliac crest. Hip circumference was determined at the widest circle of the bottom. Venous blood samples were obtained from the subjects after an overnight fasting (12 h) for the measurement of fasting blood glucose (FBG), fasting insulin (FIN), total triglyceride (TG), total cholesterol (TCHO), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C), alanine transaminase (ALT) and aspartate aminotransferase (AST). All laboratory biochemical parameters were measured in a conventional automated analyzer.

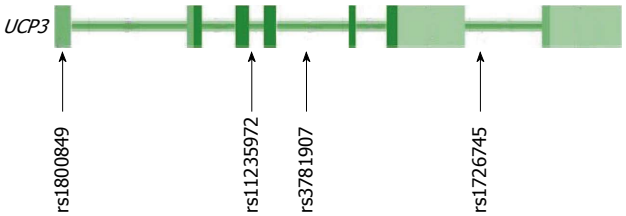

Figure 1 Locus of the human *UCP3* gene in 11q13. The uncoupling protein 3 (*UCP3*) gene consists of seven exons separated by six introns. Boxes indicate exons, while lines indicate introns and intergenic regions. Mark the polymorphism locations.

Liver ultrasound examination

Sagittal hepatic sections that encompassed longitudinal images of the right liver lobe and the ipsilateral kidney were obtained. Liver-kidney contrast with two other well-known ultrasonographic findings of fatty liver, vascular blurring and deep attenuate, enabled us to grade fatty change semi-quantitatively[15]. Ultrasound examination was carried out and blinded to laboratory values on the same equipment (GE, LOGIC 500), using a convex 3.5-5.0 MHz probe. NAFLD and healthy individuals underwent liver ultrasound examination.

DNA preparation and single nucleotide polymorphism genotyping

Using information on single nucleotide polymorphism (SNP) allelic frequencies from the website of the National Center for Biotechnology Information (NCBI) and the SNP browser software 3.0 (Applied Biosystems, Branchburg, NJ, United States), SNPs on the human *UCP3* gene with minor allele frequencies > 30% were selected. SNPs with relatively high minor allele frequencies have been shown to be very useful as genetic markers for genetic association studies. We selected four non-synonymous SNPs in the *UCP3* gene: polymorphisms of rs1726745, rs3781907, rs11235972, and rs1800849 (Figure 1). Genomic DNA was extracted from blood samples collected from each subject. Polymorphisms were genotyped using an automated platform MassARRAY (Sequenom, San Diego, CA). Polymerase chain reaction for the DNA sequence containing the target SNP was performed. The products were extended one base in SNP sites using the SNP specific primer. The products were applied into the MassARRAY SpectroCHIP array and crystallized with matrix in the chip. The crystal containing chip was moved to the mass spectrometer vacuum tube and excited using an instantaneous nanosecond (10^{-9} s) laser. The molecular of matrix absorb the radiation energy, which lead to energy accumulation causing crystal matrix sublimation, DNA molecule desorption and transformation to metastable ions.

Statistical analysis

Quantitative data with normal distribution were presented as mean ± SD. Categorical variables were expressed as a percentage and examined using the χ^2 test and Fisher's tests. Hardy-weinberg test was performed to calculate allelic frequencies using the χ^2 test. 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 OR and 95%CI for the potential risk factors of NAFLD. The statistical significance of means was estimated by independent *t* test. HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.A *P* value of < 0.05 was regarded as statistical significant. All data analysis was done using the SPSS for windows (version 13.0; SPSS, Inc., Chicago, IL, United States). Haploview software (Cambridge, MA, United States) was used to screen tag SNPs.

Table 1 Demographic and biochemical features of patients with nonalcoholic fatty liver disease and normal controls

	NAFLD (n = 250)	Controls (n = 200)	*P* value
Gender (M/F)	147/103	109/91	0.36
Age (yr)	10.78 ± 2.07	10.63 ± 2.22	0.47
Body height (cm)	148.28 ± 11.77	141.22 ± 12.91	0.00
Body weight (kg)	62.82 ± 15.17	34.72 ± 10.28	0.00
BMI (kg/m^2)	28.13 ± 3.50	17.05 ± 2.16	0.00
SBP (mmHg)	114.02 ± 11.55	91.61 ± 9.68	0.00
DBP (mmHg)	68.57 ± 8.63	66.43 ± 7.67	0.01
Waist (cm)	89.84 ± 9.74	58.07 ± 7.04	0.00
Hip (cm)	93.85 ± 8.66	70.09 ± 7.85	0.00
WHR	0.95 ± 0.06	0.83 ± 0.05	0.00
FBG (mmol/L)	4.99 ± 0.41	4.91 ± 0.38	0.04
TCHO (mmol/L)	4.40 ± 0.89	4.14 ± 0.66	0.00
HDLc (mmol/L)	1.33 ± 0.51	1.52 ± 0.30	0.00
LDLc (mmol/L)	2.53 ± 0.72	2.19 ± 0.56	0.00
TG (mmol/L)	1.41 ± 0.84	0.80 ± 0.31	0.00
ALT (mmol/L)	72.70 ± 70.16	17.14 ± 10.48	0.00
AST (mmol/L)	47.26 ± 36.61	25.22 ± 6.53	0.00
FIN (mIU/L)	20.49 ± 17.25	6.98 ± 3.59	0.00
HOMA-IR	4.57 ± 3.92	1.52 ± 0.78	0.00

Data are expressed as absolute mean ± SD. Analysis was conducted using χ^2 test and *t* test. NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic pressure; WHR: Waist-to-hip ratio; FBG: Fasting blood glucose; TCHO: Total cholesterol; HDLc: High-density lipoprotein-cholesterol; LDLc: Low-density lipoprotein-cholesterol; TG: Total triglyceride; ALT: Alanine transaminase; AST: Aspartate aminotransferase; FIN: Fasting insulin; HOMA-IR: Homeostasis model assessment-estimated insulin resistance; M: Male; F: Female.

RESULTS

The clinical features of the NAFLD and control groups are shown in Table 1. There was no significant difference in age and gender (P > 0.05). NAFLD patients showed most of the risk factors for the MetS: elevated BMI, waist-to-height ratio (WHR), BP, FBG, HOMA-IR, TG, TCHO and LDL, while decreased HDL level compared to control group.

Table 2 The genotypic distributions of the four loci in the *UCP3* gene *n* (%)

	n	Genotypes			*P* value	Allele Frequency		*P* value
		Homozygous wild-type	Homozygous mutant-type	Heterozygous mutant-type		Mutant-type	Wild-type	
rs1726745		GG	AA	AG		A	G	
NAFLD	249	40 (16.1)	102 (41.0)	107 (43.0)	0.59	311 (62.4)	187 (37.6)	0.29
Controls	200	37 (18.5)	73 (36.5)	90 (45.0)		236 (59.0)	164 (41.0)	
rs3781907		TT	CC	CT		C	T	
NAFLD	239	87 (36.4)	42 (17.6)	110 (46.0)	0.23	194 (40.6)	284 (59.4)	0.19
Controls	198	57 (28.8)	37 (18.7)	104 (52.5)		178 (44.9)	218 (55.0)	
rs11235972		GG	AA	AG		A	G	
NAFLD	236	130 (55.1)	26 (11.0)	80 (33.9)	0.03	132 (28.0)	340 (72.0)	0.28
Controls	198	90 (45.5)	16 (8.0)	92 (46.5)		124 (31.3)	272 (68.7)	
rs1800849		CC	TT	TC		T	C	
NAFLD	249	133 (53.4)	25 (10.0)	91 (36.5)	0.14	141 (28.3)	357 (71.7)	0.54
Controls	199	92 (46.2)	16 (8.0)	91 (45.7)		123 (30.9)	275 (69.1)	

NAFLD: Nonalcoholic fatty liver disease.

Table 3 Multivariate regression analysis for risk factors of nonalcoholic fatty liver disease (*n* = 449)

	B	SE	Wald	*df*	Sig	Exp (B)	95%CI for EXP (B)	
							Lower	Upper
BMI	1.98	0.77	6.67	1	0.01	7.24	1.61	32.53
HOMA-IR	1.10	0.47	5.54	1	0.02	3.01	1.20	7.55
ALT	0.05	0.06	0.54	1	0.46	1.05	0.93	1.19
TCHO	-0.64	0.88	0.53	1	0.47	0.53	0.10	2.94
rs1800849	-7.28	40192.97	0	1	1	0	0	-
rs11235972	5.15	40192.97	0	1	1	172.72	0	-
rs3781907	1.09	2.01	0.30	1	0.59	2.98	0.06	151.89
rs1726745	-0.67	4.42	0.02	1	0.88	0.51	0	2933.6

TCHO: Total cholesterol; ALT: Alanine transaminase; HOMA-IR: Homeostasis model assessment-estimated insulin resistance; BMI: Body mass index.

The distributions of the four SNPs (rs1726745, rs3781907, rs11235972 and rs1800849) obeyed the Hardy-Weinberg equilibrium in all subjects. The genotypic distributions of the four loci in the *UCP3* gene are shown in Figure 1 and Table 2. The genotype distributions of rs11235972 in the NAFLD group differed significantly from that in the control group.

When all variables were put into multivariate logistic regression analysis, higher BMI and HOMA-IR were risk factors for the development of NAFLD (Table 3). The clinical features in subjects with or without rs1800849 variation are shown in Table 4. There were no significant differences in anthropometric and biomedical variables with or without rs11235972 variation in the NAFLD and in the control groups, respectively. We found body height between the CC (142.93 ± 13.08 cm) and CT+TT (139.38 ± 12.10 cm), waist circumference between the CC (58.76 ± 6.45 cm) and CT+TT (57.00 ± 5.59 cm) and hip circumference between the CC (71.28 ± 7.84 cm) and CT+TT (69.06 ± 7.75 cm) genotypes were significantly different in the control group with and without rs1800849 variation ($P < 0.05$).

When all genotypes of the four loci were entered into haplotype analysis using Haploview, only two haplotypes' alleles *i.e.*, GC and AT frequencies were accepted for assessment between the NAFLD and control groups. However, this did not reach the significance as an independent risk factor for NAFLD (Table 4 and Figure 2).

DISCUSSION

Previous reports have demonstrated that the prevalence of NAFLD increased 10%-80% in individuals with obesity, 35%-90% in individuals with T2DM, 30%-56% in individuals with hypertension, and 26%-58% in individuals with dyslipidemia[16-18]. The prevalence of the MetS among subjects with NAFLD is 17%-36%, depending on gender and criteria used[19]. In the present study, we found that an increased risk of NAFLD was significantly associated with BMI, WHR, BP, FBG, HOMA-IR, TG, TCHO and LDL, and decreased HDL level. In agreement with these studies, Iacobellis *et al*[20] reported that a BMI evaluation may be useful in identifying those children at higher risk for disease progression. In the present study, children with NAFLD had an elevated WHR, a surrogate marker for visceral fat. Visceral fat is closely correlated with hepatic TG content, elevated ALT, liver inflammation, and fibrosis[21-23]. Central obesity is a better measure of predisposition to insulin resistance, and is more closely associated with NAFLD.

UCP3 is considered to be associated with obesity, given the role for *UCP3* in the regulation of energy and

Table 4 Demographic and biochemical features of subjects with and without rs1800849 variation (mean ± SD)

	Control			NAFLD		
	CC (n = 92)	CT+TT (n = 107)	P value	CC (n = 133)	CT+TT (n = 116)	P value
Gender (M/F)	48/44	60/47	0.582	103/30	90/26	0.979
Body height (cm)	142.93 ± 13.08	139.38 ± 12.10	0.048	148.82 ± 12.50	147.83 ± 10.82	0.510
Body weight (kg)	35.65 ± 9.69	33.29 ± 8.51	0.070	64.42 ± 16.86	61.14 ± 12.74	0.083
BMI (kg/m^2)	17.11 ± 2.00	16.86 ± 1.80	0.358	28.45 ± 3.75	27.79 ± 3.17	0.141
SBP (mmHg)	91.59 ± 8.89	91.29 ± 9.80	0.824	113.32 ± 11.74	114.84 ± 11.36	0.301
DBP (mmHg)	66.71 ± 7.11	66.13 ± 8.16	0.595	68.27 ± 9.13	68.91 ± 8.08	0.561
Waist (cm)	58.76 ± 6.45	57.00 ± 5.59	0.040	90.78 ± 10.58	88.87 ± 8.57	0.123
Hip (cm)	71.28 ± 7.84	69.06 ± 7.75	0.047	94.31 ± 9.51	93.47 ± 7.64	0.483
WHR	0.83 ± 0.05	0.83 ± 0.04	0.833	0.95 ± 0.07	0.95 ± 0.05	0.559
FBG (mmol/L)	4.91 ± 0.35	4.92 ± 0.40	0.888	5.02 ± 0.44	4.96 ± 0.36	0.245
TG (mmol/L)	4.11 ± 0.65	4.17 ± 0.67	0.519	4.32 ± 0.82	4.48 ± 0.96	0.165
HDL (mmol/L)	1.48 ± 0.29	1.55 ± 0.30	0.128	1.33 ± 0.58	1.35 ± 0.40	0.743
LDL (mmol/L)	2.18 ± 0.52	2.19 ± 0.58	0.950	2.50 ± 0.70	2.56 ± 0.75	0.457
ALT (mmol/L)	16.85 ± 6.44	16.48 ± 8.92	0.740	54.37 ± 2.07	51.43 ± 2.28	0.574
AST (mmol/L)	23.90 ± 5.54	26.07 ± 6.56	0.013	47.53 ± 36.24	46.91 ± 37.35	0.896
FINS	7.20 ± 3.34	6.62 ± 3.35	0.226	17.13 ± 2.11	14.86 ± 2.12	0.140
HOMA-IR	1.58 ± 0.75	1.45 ± 0.74	0.231	3.80 ± 2.14	3.27 ± 2.12	0.117

Analysis was conducted using t test. Statistically significant differences between groups are shown in bold. NAFLD: Nonalcoholic fatty liver disease; M: Male; F: Female; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic pressure; WHR: Waist-to-hip ratio; FBG: Fasting blood glucose; HDL: High-density lipoprotein-cholesterol; LDL: Low-density lipoprotein-cholesterol; TG: Total triglyceride; ALT: Alanine transaminase; AST: Aspartate amino-transferase; FIN: Fasting insulin; HOMA-IR: Homeostasis model assessment-estimated insulin resistance.

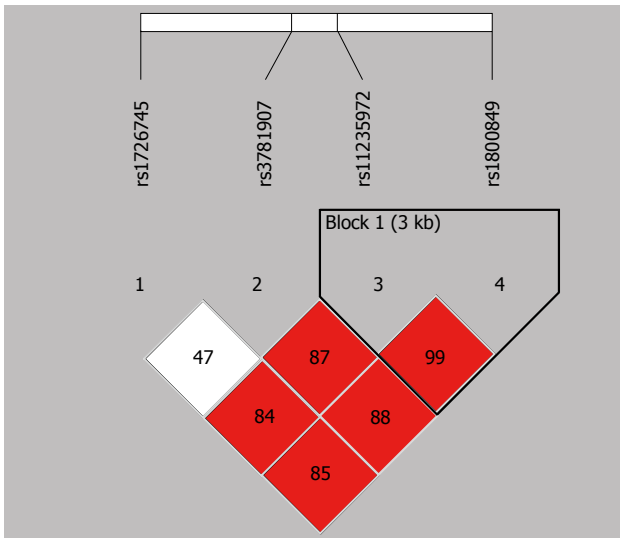

Figure 2 Linkage Disequilibrium mapping around the uncoupling protein 3 between nonalcoholic fatty liver disease and control groups.

Table 5 Haplotype frequencies of rs1800849, rs11235972, rs3781907, rs1726745

Block	Frequencies		χ^2	P value
	NAFLD	Control		
GC	0.712	0.682	0.901	0.343
AT	0.282	0.307	0.702	0.402

NAFLD: Nonalcoholic fatty liver disease.

lipid metabolism. However, the effect of genetic polymorphisms in *UCP3* on the pathogenesis of NAFLD has not been clearly documented. In our study, the GG genotype distribution of rs11235972 in the NAFLD group differed significantly from the control group. The results from the literature are controversial. Yoon *et al*[24] genotyped 6 polymorphisms of *UCP3* among overweight female subjects (n = 458), and genetic effects on BMI and changes after a very low calorie diet were examined. They found that several polymorphisms in the *UCP2-3* gene cluster showed associations with changes in BMI and fat mass. Hamada *et al*[25] determined whether the *UCP3*-55 C/T SNP was associated with obesity according to the criteria for Japanese (BMI ⩾ 25 kg/m^2) and serum HDL-C levels in the general population. Subjects with the T/T genotype had significantly higher HDL-C levels than those with the C/C genotype or C/T genotype. Furthermore, subjects with the T/T genotype had a significantly lower BMI than those with the C/C genotype[25]. Salopuro *et al*[26] found that the *UCP3* gene variant rs3781907 was associated with increased serum TCHO and LDL cholesterol levels. The rs1726745, rs11235972 and rs1800849 variants in the *UCP3* gene are associated with serum total and LDL-cholesterol at baseline. However, de Luis *et al*[27] did not demonstrate an association between the -55CT polymorphism of the *UCP3* gene and fat distribution in obese patients. There might be true variability in the association among different populations, particularly different ethnic groups.

A common promoter polymorphism has also been identified in the *UCP3* gene (rs1800849), a rare allele associated with obesity in a recessive manner in several studies[10-12]. Moreover, the rs1800849 allele is associated with a higher WHR[28], but no association between rs1800849 and WHR existed in the current study. We showed a significant difference in height, and waist and hip circumference between the CC (mutant type group) and CT+TT group with and without rs1800849 variation (Table 5).

One limitations in this study was that we used abdominal ultrasonography to diagnose NAFLD, although validation ultrasonography has a sensitivity of 91.7% and a specificity of 100%[29]. The diagnosis of NAFLD was based on ultrasound and was not confirmed by liver biopsy due to the invasive procedure usually not initially performed and ethical considerations. Thus, surrogate markers are commonly used, such as transaminases and imaging techniques. Computed tomography is more specific but is not used for screening of fatty liver in obese children. Magnetic tomography is more useful in adults and not appropriate for children due to ionizing radiation. Magnetic resonance imaging and 1H-MRS have the greatest accuracy to determine hepatic fat content, but are rarely used due to high costs. A higher prevalence of the rs11235972 GG genotype was noted in the NAFLD group compared with the control group; no differences were observed for the other SNPs. BMI and HOMA-IR increased the risk of NAFLD. Moreover, no increased risk for developing NAFLD was found to be associated with the rs1800849 variant based on multivariate analysis. A significant difference in height, and waist and hip circumference between the CC and CT+TT group with and without rs1800849 variation was demonstrated.

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COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic condition characterized by abnormal lipid deposition in hepatocytes in the absence of excess alcohol intake. There are few population-based prevalence studies of pediatric NAFLD. Elucidation of genetic factors that predispose an individual to NAFLD may lead to development of non-invasive biomarkers for the early diagnosis of NAFLD.

Research frontiers

Uncoupling protein 3 (*UCP3*) is considered to be associated with obesity, given the role for *UCP3* in the regulation of energy and lipid metabolism. However, the effect of genetic polymorphisms in *UCP3* on the pathogenesis of NAFLD has not been clearly documented.

Innovations and breakthroughs

Theoretically, many variations in candidate genes related to MetS may contribute to the pathogenesis of NAFLD. This is the first study to report that there significant difference of body height, waist and hip circumference between CC (mutant type group) and CT+TT group with and without rs1800849 variation were found.

Applications

This study confirmed the hypothesis that polymorphisms of the *UCP3* are associated with the occurrence of NAFLD. These variations could be useful for the diagnosis and/or prognosis of NAFLD.

Terminology

UCP3 gene is located on chromosome 11q13. *UCP3* is a mitochondrial anion carrier protein with a highly selective expression in skeletal muscle, a major site of thermogenesis in humans, which makes an attractive target for studies into the regulation of body weight.

Peer review

The authors examined the polymorphisms of the *UCP3* gene associated with the occurrence of NAFLD. Higher rs11235972 GG genotype prevalence has been observed in the NAFLD group compared with the control group. There significant difference of body height, waist and hip circumference between CC (mutant type group) and CT+TT group with and without rs1800849 variation were found. This is an interesting manuscript about NAFLD in children and *UCP3* polymorphisms. The manuscript has adequate methodology and is good written.

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