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Effect of the variation of exon 20 of leptin receptor gene on lipid metabolism in children with obesity

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Abstract: Objective To study the relationship of the variation of the exon 20 of leptin receptor (*LEPR*) gene with lipid metabolism and fat distribution in children with obesity. **Methods** One hundred and two children with simple obesity (Obesity group) and 81 healthy children (Control group) were enrolled in this study. Polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP) and polyacrylamide gel electrophoresis were used to analyze the variation of the exon 20 of *LEPR* gene. The serum levels of triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) were measured. Body mass index (BMI) and fat percentage (% fat) were calculated. **Results** Three genotypes of the exon 20 of *LEPR* gene were detected: G/G, G/A and A/A. The frequency of the gene variation at 3 057 nucleotide G→A transversion was significantly higher in the Obesity group when compared with the Control group ($P<0.05$). The levels of serum TG (1.8 ± 0.5 mmol/L vs 1.0 ± 0.4 mmol/L, $P<0.01$), BMI (33 ± 5 kg/m^2 vs 25 ± 4 kg/m^2, $P<0.05$) and % fat (30 ± 8 vs 20 ± 3, $P<0.01$) in obese children with A/A genotype were higher than those of the G/G genotype ones, but the level of serum HDL was lower than that of the G/G genotype patients (1.08 ± 0.23 mmol/L vs 1.38 ± 0.22 mmol/L, $P<0.01$). As to the G/A genotype patients, only their serum TG levels were higher than those of the G/G genotype ones (1.6 ± 0.4 mmol/L vs 1.0 ± 0.4 mmol/L, $P<0.05$). The electrocardiography showed the changes of ST segment and T wave in parts of A/A genotype patients with hyperlipoidemia. **Conclusions** There were polymorphisms in children with obesity and these may affect the lipid metabolism and the fat distribution. The study provided theoretic evidence for the early interference of childhood obesity.

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Key words: Leptin receptor; Polymorphism, genetic; Lipid metabolism; Obesity; Child

瘦素受体基因 20 外显子突变对儿童脂质代谢影响的临床研究

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[**摘 要**] **目的** 探讨瘦素受体基因(*LEPR*)第 20 外显子突变对脂质代谢的影响及肥胖儿童基因型与血脂的关系。**方法** 用聚合酶链反应限制性片段长度多态性(PCR-RFLP)方法及聚丙烯酰胺凝胶电泳分析 20 外显子的基因突变频率,并测定单纯型肥胖儿童(102 例)和健康儿童(81 例)血清中甘油三脂(TG)、总胆固醇(TC)、高密度脂蛋白(HDL)和低密度脂蛋白(LDL)水平。两组分别测量身高、体重,计算体重指数(BMI)及脂肪百分比。**结果** 肥胖儿童瘦素受体基因的 20 外显子经 PCR-RFLP 及聚丙烯酰胺凝胶电泳分析,检测出 3 种基因型 G/G, G/A 和 A/A 型。肥胖儿童 20 外显子 3 057 位 G→A 突变频率较健康儿童增高($P<0.05$)。A/A 基因型的肥胖儿童其血清 TG (1.8 ± 0.5 mmol/L vs 1.0 ± 0.4 mmol/L, $P<0.01$)、BMI (33 ± 5 kg/m^2 vs 25 ± 4 kg/m^2, $P<0.05$) 水平和脂肪百分比 (30 ± 8 vs 20 ± 3, $P<0.01$) 均明显高于 G/G 基因型者,而血清 HDL 水平则低于后者(1.08 ± 0.23 mmol/L vs 1.38 ± 0.22 mmol/L, $P<0.01$)。G/A 型肥胖儿童,除其血清 TG 浓度高于 G/G 基因型者外(1.6 ± 0.4 mmol/L vs 1.0 ± 0.4 mmol/L, $P<0.05$),余各项指标均与另外两种基因型无明显差别。心电图检查显示部分 A/A 型血脂增高儿童有 ST 段和 T 波改变。**结论** 单纯型肥胖儿童瘦素受体基因第 20 外显子存在基因多态性,

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且该多态性明显影响肥胖儿童的脂质代谢及体脂分布。该研究为临床上开展对肥胖儿童的早期干预提供了理论依据。

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[关 键 词] 瘦素受体;多态性,基因;脂质代谢;肥胖症;儿童

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With the increase of living standards, the incidence of childhood obesity is increasing, resulting in a poor physiopsychic development of children[1]. The incidence of childhood obesity has increased by nearly 40% in the United States in recent years[2]. Therefore, more and more researchers are focusing on the pathogenesis of obesity in order to prevent this disorder.

Leptin receptor (*LEPR*) gene is closely related to the human obesity, which locates at the human chromosome 1p31. It is 5.1 kb in size, contains 20 exons and 19 introns and encodes 1 165 amino acids[3]. Its gene product is leptin receptor, which distributes mainly at hypothalamus. It binds with leptin and adjusts body energy metabolism through signal conduction pathway.

This study investigated the relationship between the *LEPR* gene exon 20 mutation and lipid metabolism in childhood obesity.

Subjects and methods

Subjects

Obese children from pediatric out-patient and in-patient departments or identified from physical examination were enrolled. WHO recommended child body height / body weight standards were applied as the normal control criterion. Obesity was defined as > 20% overweight compared with standard body weight (calculated according to body height). One hundred and two children were recruited into the Obesity group (60 males and 42 females), aged between 6-14 years (mean 9.86 ± 2.28 years). Eighty-one healthy children (50 males and 31 females) aged between 6-13.5 years (mean 9.25 ± 1.98 years) matched the Obesity group in age, region, economical condition and living conditions were used as the Control group. Body heights and body weights in both groups were measured under the same conditions. Body mass index (BMI) and fat percentage (% fat) were calculated according to equations: BMI = body weight/(body height)^2 (kg/m^2); % fat = 1.2 × BMI + 0.23 × age (year) −16.2 (male), or = 1.2 × BMI + 0.23 × age (year) −5.4 (female)[4].

Measurement of blood lipid levels

Total cholesterol (TC) concentration was measured with the cholesterol oxidase- peroxydase endpoint method. Triglyceride (TG) concentration was measured by glycerophosphoric acid oxidase-peroxydase endpoint method. High density lipoprotein-cholesterol (HDL-C) concentration was measured by the direct assay method. The concentrations of low density lipoprotein-cholesterol (LDL-C), Apo A1 and Apo B100 were measured by the immune turbidimetry method. The kits used in the tests were provided by the Beijing Lideman Biochemical Technique Ltd Co. These measurements were done by the same automatic biochemistry analyzer (AU600 Olympus, Japan).

Extraction of DNA

After 12 hour of fasting, 7 mL peripheral venous blood was collected from the subjects. Five mL blood anticoagulated with 1 mL 13.43 mM EDTA-Na_2 was thoroughly mixed with 9 × STMT solution (8% sucrose, 10 mM Tris-HCl, 5 mM $MgCl_2$, 1% Triton X-100), and placed at 4 ℃ for 30 minutes. Then the samples were centrifuged at 4 000 rpm for 15 minutes. The precipitate was washed with 154 mM NaCl twice before proteinase-K SDS treatment. Finally, the genomic DNA was extracted by phenol-chloroform and dehydrated alcohol extraction. The concentration and purity of genomic DNA were measured with UV spectrophotometer (Daojin, UV-15). Serum was prepared from the remaining 2 mL of blood and stored at -86 ℃ for the measurement of blood lipid levels.

Polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP)

PCR primers were designed according to the *LEPR* gene sequence reported by Matssuoka[4] (Forward: 5′-ACT GTG GTC TCT CTA CTT TC-3′, Reverse: 5′-CCA TGA GCT ATT AGA GAA AGA

ATC CGT CAA-3′). The PCR reaction solution (25 μL) consisted of genomic DNA 200 ng, 10 × buffer 2.5 μL, $MgCl_2$ 1.2 mM, dNTPs 0.24 mM each, primers 0.75 μM each, and Taq polymerase (Promega) 0.8 U. Amplification was carried out in a PE-9600 Amplifier. The PCR reaction began with an initial denaturation at 95 ℃ for 8 minutes, followed by 30 cycles of denaturation at 94 ℃ 60 seconds, annealing at 72 ℃ 50 seconds, extension at 72 ℃ 50 seconds, and then a final extension at 72 ℃ for 8 minutes. The PCR product was 276 bp in size. PCR products were examined by 2.5% agarose gel electrophoresis with ethylene dibromide (EB) stain. Enzyme digestion reaction was carried out in a 25 μL solution, which consisted of 2 μL PCR products, Hinc II 12 μL (10 U/μL, Promega), 10 × buffer B 2.5 μL, at 65 ℃ for 4 hours. Neutral polyacrylamide gel (8%, degree of cross linking 29∶1, ionic strength 1 × TBE) was prepared according to the routine method. Electrophoresis was carried out under room temperature for 4 hours at 200 V. Results were analyzed using silver staining according to the published document [5].

Individual genotype identification

The Hardy-Weiberg equilibrium test was carried out to confirm the representation of samples and genotypic frequencies and allelic gene frequencies were calculated out. Variables with normal distribution were expressed as $\bar{x} \pm s$ and those with non-normal distribution were conversed into a natural logarithm. A Chi-square test was performed to compare the numeration data among multi-groups. ANOVA analysis and q test were used for the comparison of measurement data.

Ultrasonic cardiogram and electrocardiogram (ECG) examination

Ultrasonic cardiogram, electrocardiogram examination or postexercise ECG were performed on 16 obese children with A/A genotype and with TG content > 1.81 mmol/L, TC content > 4.33 mmol/L and LDL content > 2.18 mmol/L.

Results

Genotype analysis

LEPR had three kinds of results of Hinc II enzyme digestion: 1) Homozygote G/G had 2 digestion sites and resulted in 3 fragments (28 bp, 75 bp, 173 bp); 2) Homozygote A/A had only 1 digestion site and resulted in 2 fragments (75 bp, 201 bp); and 3) Heterozygote G/A had 4 fragments (28 bp, 75 bp, 173 bp, 201 bp). The genotype frequencies of G/G, G/A and A/A in healthy and obese children are shown in Table 1. Compared with the Control group, the frequency of the gene variation at 3 057 nucleotide G→A transversion was significantly higher in the Obesity group ($\chi^2 = 7.489$, $P < 0.05$).

The relationship of the *LEPR* gene exon 20 mutation with lipid metabolism and fat distribution

The relationship of the *LEPR* gene exon 20 mutation with lipid metabolism and fat distribution in children with obese is listed in Table 2. In the obesity patients with A/A genotype, the serum TG level, BMI and % fat were significantly higher than those of G/G genotype ones ($q = 4.74$, $P < 0.01$), whereas the HDL level was lower ($q = 3.97$, $P < 0.01$). As to obesity children with G/A genotype, although their serum TG levels were significantly higher than patients with G/G genotype ($q = 3.18$, $P < 0.05$), there were no significant differences in other parameters compared with the G/G and A/A genotype patients. These results indicated that the 3 057 G→A mutation of *LEPR* gene in children with obesity could affect the blood lipid metabolism, which resulted in higher serum TG and lower HDL levels, and therefore affected the body fat distribution.

Table 1 Comparison of genotype frequency between healthy and obese children

Group	n	Genotype frequency			Allele frequency	
		GG(%)	GA(%)	AA(%)	G(%)	A(%)
Control	81	24 (29.6)	35 (43.2)	22 (27.1)	83 (51.2)	79 (48.7)
Obesity	102	14 (13.7)	30 (29.4)	58 (56.8)	58 (28.5)[a]	146 (71.5)[a]

a compared with the Control group, $\chi^2 = 7.489$, $P < 0.05$

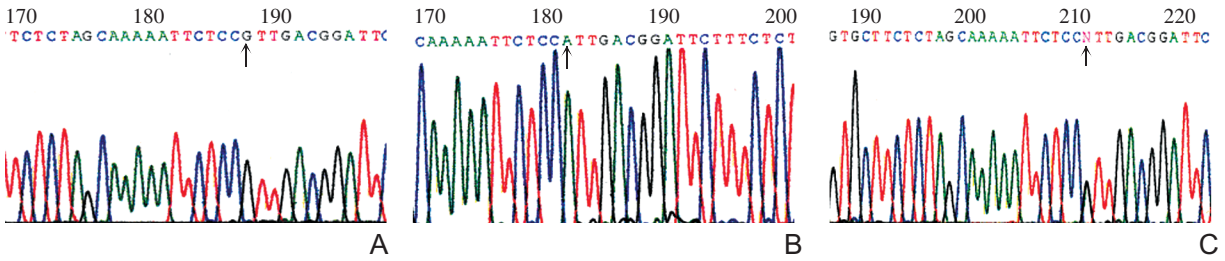

Figure 1 Results of genotype sequencing of *LEPR* gene exon 20

A: Healthy control; B: Homozygote after mutation; C: Heterozygote after mutation

Table 2 Effect of *LEPR* gene variation on the blood lipid level and the body fat distribution

Genotype	TG(mmol/L)	TC(mmol/L)	HDL(mmol/L)	LDL(mmol/L)	BMI(kg/m^2)	% fat
G/G	1.0 ± 0.4	3.8 ± 0.3	1.4 ± 0.2	1.8 ± 0.5	25 ± 4	20 ± 3
G/A	1.6 ± 0.4[a]	4.0 ± 0.5	1.2 ± 0.2	2.1 ± 0.4	30 ± 5	27 ± 7
A/A	1.8 ± 0.5[b]	4.3 ± 0.8	1.1 ± 0.2[b]	2.2 ± 0.6	33 ± 5[b]	30 ± 8[b]
F	5.699	1.923	3.79	1.11	6.68	3.89
P	< 0.01	> 0.05	< 0.05	> 0.05	< 0.01	< 0.05

Compared with the G/G genotype a $P < 0.05$, b $P < 0.01$

Genotype sequencing

The results of PCR products sequencing are shown in Figure 1. The comparison of these results with Genebank data (U 59 263) confirmed the consistency of the PCR product with *LEPR* gene exon 20. Moreover, mutation could be detected at the 3 057th nucleotide, i. e. a G→A mutation, therefore 3 genotypes could be identified: homozygote G/G, heterozygote G/A and homozygote A/A.

Recordings of ultrasonic cardiogram and ECG in children with obesity

Thickening, echo enhancement, irregular and unsmooth changes in the coronary artery wall and lumen stenosis of coronary artery were observed in 3 of the 16 children with severe obesity. Abnormal ventricular wall movements and decrease of ejection fraction were noticed in 2 patients. ST-T changes at different degrees were observed in 11 children, mainly manifesting as lowering of ST segment and changes of T wave. Postexercise ECG showed lowering of ST segment (0.03 – 0.04 mV) in 4 out of 9 patients. Paroxysmal tachycardia was induced in 2 patients. T wave changes were observed in other patients, mainly presenting with being shorter than 1/10 R wave of the same lead.

Discussion

Yiannakouris et al[6] studied 118 obese patients' *LEPR* gene and found 3 polymorphisms, i. e. Lys-109Arg, Gly-223Ser and Lys-656Asn. It was reported that the *LEPR* gene Lys109Arg polymorphism was related with the development of obesity and the lipid distribution of patients, while the other 2 polymorphisms were unrelated. In 1997, Matssuoka[5] analyzed the 2 to 20 exons of *LEPR* gene and identified 7 nucleotides variations, i. e. Lys-109Arg, Gln-223Ser, Ser-343Ser, Ser-492Thr, Lys-656Asn, Ala-976Asp and Pro-1 019Pro. In this study the 20th exon of *LEPR* gene was chosen to investigate the relationship between the variation of the *LEPR* gene and lipid metabolism as well as the fat distribution in children with obesity. The results indicated that: 1) The 3 057 G→A mutation in *LEPR* gene was detected in children with simple obesity, with the frequency of 86. 3%, which was consistent with others' report[5]; 2) The mutation at the *LEPR* gene 20th exon could affect the blood lipid metabolism of children with simple obese. A higher serum TG level and lower HDL level were observed in obese children with A/A genotype compared with G/G genotype. While in obese children with G/A genotype, only the serum TG level was significantly higher than that in children with G/G genotype. 3) The mutation of 20th exon could affect the fat distribution of children with obesity. In simple obese children with G/G or G/A genotype, the BMI and % fat were significantly higher than those of patients with A/A genotype. The possible mechanism of the difference is as follows. The structure of the gene product (leptin receptor) changed as the *LEPR* gene mutated and therefore could not bind with leptin. It resulted in the absence of the biologic effects of leptin (inhibition of appetite, promotion of energy consumption and promote

the lipid tissue degeneration[7]), and led to the abnormality of lipid metabolism and the accumulation of adipose tissue.

Regarding the relationship between obesity and coronary heart disease (CHD), it was reported that thickening and incomplete of sclerous tissues of coronary artery wall could be observed by autopsy in under 5 year-old children with simple obesity[8,9]. Results of this study indicated that different degrees of coronary artery damage could be noticed in children with severe obesity with hyperlipoidemia. Therefore, prevention of atherosclerosis disease should be initiated from children with obesity[10], especially those with increased ε4 allelic frequency, which is the genetic susceptibility of CHD. Therefore, it was very important to detect the genotype of childhood obesity, make an early diagnosis of hyperlipoidemia, and carry out effective interference measures for the prevention of early CHD. The results of this study indicated that gene polymorphism of *LEPR* gene 20th exon could be detected in children with simple obesity. This polymorphism could significantly affect the lipid metabolism and body fat distribution. The study provided theoretic evidence for the early interference of childhood obesity to reduce the incidence of hyperlipoidemia, CHD, diabetes mellitus in adults.

[**References**]

[1] Li T-Y, Ding Z-Y, Yang X-Q, He X-H. Children are the future of the world: a brief introduction to the 23rd World Congress of Pediatrics (in Chinese) [J]. Chin J Pediatr, 2004, 42(12): 942-944.

[2] Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The continuing epidemic of obesity in the United States [J]. JAMA, 2000, 284(13): 1650-1651.

[3] Smith JC. The current epidemic of childhood obesity and its implications for future coronary heart disease[J]. Pediatr Clin N Am, 2004, 51(6): 1679-1695.

[4] Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas [J]. Br J Nutr, 1991, 65(2): 105-114.

[5] Matssuoka N, Ogawa Y, Hosoda K. Human leptin receptor gene in obese Japanese subject: Evidence against either obesity-causing mutation or association of sequence variants with obesity[J]. Diabetologia, 1997, 40 (10): 1204-1210.

[6] Yiannakouris N, Yannakoulia M, Melistas L. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability[J]. J Clin Endocrinol Metab, 2001, 86 (9): 4434-4439.

[7] Quinton ND, Lee AJ, Ross RJ. A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women[J]. Hum Genet, 2001, 108(3): 223-226.

[8] Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and dibetes in the United States[J]. JAMA, 2001, 286(10): 1195-1200.

[9] Dong C, Wang S, Li WD, Li D, Zhao H, Price RA. Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity[J]. Am J Hum Genet, 2003, 72(1): 115-124.

[10] Sabharwal NK, Lahiri A. Role of myocardial perfusion imaging for risk stratification in suspected or known coronary artery disease [J]. Heart, 2003, 89 (11): 1291-1297.

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