Iranian Journal of Basic Medical Sciences

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Correlational studies on insulin resistance and leptin gene polymorphisms in peritoneal dialysis patients

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Oct 20,2014
Accepted: Feb 1, 2015

Keywords:
Insulin resistance
Leptin
Peritoneal dialysis
Polymorphisms

ABSTRACT

Objective(s): The aim of the study was to investigate the relationship between insulin resistance (IR) and leptin (LEP) gene polymorphisms in peritoneal dialysis (PD) patients.

Materials and Methods: From July 1, 2011 to August 1, 2011, patients who received chronic PD were chosen and divided into three groups (DM, high HOMR-IR, and low HOMR-IR). Two PCR products of LEP were sequenced and aligned and the distribution of polymorphisms was analyzed using $\chi 2$ analysis. In addition, serum leptin level, PD conditions, and biochemical parameters according to different genotype of G-2548A and A19G were statistically analyzed (P-value<0.05). The relationship between LEP gene polymorphisms and prognosis was explored.

Results: Totally 157 patients with average age of 55±15 years old were chosen. Distribution of genotype frequencies was complied with Hardy-Weinberg equilibrium. Leptin level and BMI (body mass index) of the GG genotype of G-2548A were higher than that of GA or AA. The fasting glucose, cholesterol, etc. of AA genotype were lower, and the nPCR was higher than the two other genotypes. Serum leptin level and BMI of AA genotype of A19G was higher than GA and GG genotypes; meanwhile, fasting blood glucose of that genotypes was the highest. In addition, survival rate of AA group of A19G was very low.

Conclusion: The G-2548A and A19G polymorphisms were correlated with serum leptin level and IR. Leptin A19G polymorphism may be prognostic for PD patients. This study may facilitate early intervention for IR in PD patients.

► *Please cite this article as:*
Cao L, Mou Sh, Fang W, Qi Ch, Chang X, Gu L, Qian J, Ni Zh. Correlational studies on insulin resistance and leptin gene polymorphisms in peritoneal dialysis patients. Iran J Basic Med Sci 2015; 18:878-886.

Introduction

Human leptin (LEP) gene is located on chromosome 7q31 (1). So far, LEP gene polymorphism is found widely distributed in the promoter, exons, and introns of the gene. Currently, studies on the human LEP gene polymorphisms suggested that these polymorphisms were mainly correlated with obesity, prostate cancer, and other diseases (2-5).

Based on comprehensive study of relevant reports, total of 10 mutations in the exon and intron regions were found. Of which, G19A, Gln25Gln, Argl05Trp, and A133 (deletion) were found to have correlations with obesity occurrence (6, 7). Besides, G19A mutation can lead to impaired glucose regulation under normal conditions (8), but not the other six mutations; furthermore, it may also affect the leptin concentrations (6). In addition, eight kinds of mutations in the promoter region of LEP were discovered, among which, G-2548A, C-1823A/T, C-633T, and C-188A were found to have a significant correlation with obesity (9-12).

Particularly, -2548 G allele has frequency of 49% to 75%, and is related to body mass index (BMI), waist circumference, hip circumference, and plasma leptin concentrations (total or free leptin concentrations) of healthy people, diabetic, and prostate cancer patients (10). Furthermore, Ren *et al* (13) found that frequency of -2548 A allele in type 2 diabetes was higher than normal, and the plasma levels of leptin and insulin in patients with AA genotype were significantly lower than those with GA or GG genotypes; meanwhile, insulin resistance index (IRI) of GG was significantly higher than AA or GA. Relationship between leptin and IR determined by homeostasis model assessment (HOMA) showed that patients carrying the C allele have higher IRI (14).

Our recent study (published online) indicated that in peritoneal dialysis (PD) patients, peritoneal transport type, the daily glucose usage, glucose uptake are not correlated with leptin levels or IR degree (15). It suggests that even exposing to the same dose of

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glucose, PD patients showed different susceptibility to glucose tolerance. Whether different glucose tolerance in PD patients is correlated with different genetic characteristics of them is not clear. Therefore, this study was performed to detect leptin genotypes in PD patients to facilitate early intervention by the early detection and better prognosis for high-risk individuals with higher baseline leptin and insulin.

Materials and Methods

Subjects

During July 1, 2011 to August 1, 2011, patients that received chronic PD in Renji Hospital peritoneal dialysis center were recruited to this study. Patients who received PD for at least 3 months were selected. Ethics approval was granted from the Ethics Committee of Renji Hospital.

Groups

Based on the median of homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated as: HOMA-IR= fasting blood glucose (mmol/l) × fasting insulin (μmol/ml)/22.5, patients were evenly divided into two groups. The median of HOMA-IR was 2.05; therefore patients with HOMA-IR higher than 2.05 were classified into high (H) group (which is also the IR group) and those with HOMA-IR lower than 2.05 were entered in L group.

Patients with type 2 diabetes (diagnosed before dialysis) and treated with PD during the same period were selected as diabetes mellitus (DM) group.

Specimens' collection

Before the peritoneal equilibration test (PET), peripheral blood of all selected patients was drawn in the morning. About 2 ml of whole blood were placed in tubes containing citrate as anticoagulant and were stored at 4 ˚C. Blood DNA was isolated with human genomic DNA extraction kit (Promega, USA).

Polymorphic gene sequencing

After the purification of PCR products of blood DNA, the promoter and exon regions of the LEP gene were sequenced. Sense and antisense primers of rs7799039 (-2548A/G) and rs2167270 (A19G) were 5-GCCTTTCTAAGCCAAGCAAA-3, 5-TTCCTGCAACATC TCAGCAC-3 and 5-GGGACATCAAGGATTTCTCG- 3, 5-GCCAAGAAAGACCAGCAGAG-3, respectively. The cycling conditions for them were at 95 ˚C for 5 min, followed by 20 cycles at 95 ˚C for 30 sec, annealing at 60 ˚C for 60 sec, extensions at 72 ˚C for 180 sec, and with a final elongation step at 72 ˚C for 10 min, finally stored at 10 ˚C.

Later on, using the ABI3730 automated DNA capillary sequencer, the DNA sequence was determined and then was compared with ClustalW software and artificial verification. The single nucleotide polymorphism (SNP) locus was finally determined.

Besides, Hardy-Weinberg equilibrium (HWE) testing, which is a genetic approach for evaluation of genetic characteristics of populations, was performed. The distribution of -2548A/G and A19G polymorphisms and those around them among groups was tested using $\chi 2$ analysis.

General conditions of patients

Patient's general conditions including age, sex, height, weight, and blood pressure were detected and recorded. Besides, subjective global assessment (SGA) of nutrition analysis was conducted based on BMI, diet situations, etc.; subcutaneous fat (skinfold thickness) over the left triceps and the mid-arm muscle circumference (16) and edema in the sacral region and ankles, which were all ranked as A, B, and C grade. A was no malnutrition, while B was moderately malnourished, and C was severe malnutrition. In addition, definition and calculation of the metabolic syndrome (MS) were performed following the standard of Chinese Diabetes Society in 2004.

In addition, serum creatinine (SCr), blood leukocyte, hemoglobin, albumin (Alb), calcium, phosphorus, parathyroid hormone (PTH), high sensitive C-reactive protein (hsCRP), fasting insulin (FINS), and glycated albumin (GA) of patients were determined at the outset.

Dialysis related indicators determination

24 hr urine and dialysate, dialysate glucose concentrations, exchange volume and frequency, dialysate calcium concentration, and the dwell time were performed. Besides, concentrations of creatinine (Cr) and blood urea nitrogen (BUN) in blood, urine, and peritoneal effluent, as well as glucose concentration in blood, and peritoneal effluent were detected. The total weekly urea clearances (Kt/V), renal Kt/V, total weekly creatinine clearance (WCcr), and the renal Ccr were calculated by the urea kinetic model (UKM) as: Kt/V=7× [D/Purea ×volume of dialysis fluid drained (L) + U/Purea×urine volume]. The value of the normalized protein catabolic rate (nPCR) and PCR was calculated as follows: PCR=5420×G/V×2.8+0.17+ blood urea nitrogen (BUN/Ti) of total urine volume × 2.8; nPCR (g/kg/day) = PCR/ normal weight.

Detection of the leptin level of patients

Based on the double antibody sandwich ABC-ELISA method, the concentration of leptin was determined with the human leptin (STH01090) ELISA kit (Shanghai Sunteam Biotech, Inc. Shanghai, China). The anti-human leptin antibody conjugated with horseradish peroxidase (HRP) and the tetramethylbenzidine (TMB) substrate were used and the optical density value at 450 nm of leptin was detected.

Table 1. Baseline characteristics of all peritoneal dialysis patients

Variable	Total	Diabetes mellitus group	High group IR>2.05	Lower group IR<2.05
Age (year)	55.24±15.23	61.15±12.97###	58.44±15.50###	49.63±14.11
Amount (male %)	157（79）	26 (17)	66(28)	65(34)
Height (cm)	162.78±8.05	162.46±8.14	161.30±7.67#	164.42±8.21
Body mass index (kg/m^2)	23.28±3.62	24.14±3.45##	24.26±3.64###	21.95±3.26
Systolic blood pressure (mmHg)	100.62±15.00	100.05±12.27	96.54±14.20###	104.99±15.76
Subjective global assessment				
Normal (%)		16 (61.5)	48 (72.7)	49 (75.4)
Moderate malnutrition (%)		9 (34.6)	17 (25.8)	14 (21.5)
Severe malnutrition (%)		1 (3.8)	1 (1.5)	2 (3.1)
Dialysis age (month)	36.07±28.98	33.69±24.11	36.29±29.71	36.80±30.35
Normalized protein catabolic rate	0.83±0.19	0.77±0.15#	0.80±0.18#	0.88±0.21
Metabolic syndrome (%)	76 (48.7)	19 (73.1)	42 (63.6)	15 (23.1)
Number of factors	2.44±0.98	2.88±0.77	2.86±0.84	1.83±0.88
Dialysate volume (l/day)	7.26±1.27	7.46±0.90	7.16±1.36	7.3±1.30
Dialysate calcium concentration				
1.25 (%)		17 (65.4%)	33 (50%)	33 (50.8%)
1.75 (%)		9 (34.6%)	33 (50%)	32 (49.2%)
Glucose intake (g/day)	65.10±17.67	69.27±10.87	64.08±15.94	64.48±21.17
Glucose absorption (g/day)	38.38±12.26	40.18±8.06	37.84±11.94	38.20±13.95
Laboratory index				
WBC（×10^9/l）	8.00±2.82	8.45±2.82	8.65±3.26	7.19±2.09
Hemoglobin(g/l)	111.44±19.39	114.96±17.14	112.68±21.53	108.78±17.85
Calcium (mmol/l)	2.30±0.21	2.28±0.25	2.34±0.23	2.27±0.18
Phosphorus (mmol/l)	1.57±0.45	1.50±0.43	1.58±0.47	1.62±0.43
Parathyroid hormone (pg/ml)	400.63±376.07	341.03±338.60	401.87±353.88	423.21±413.28
Albumin (g/l)	37.84±4.71	37.02±6.43	38.26±4.63	37.75±3.94
Cholesterol (mmol/l)	5.34±1.19	5.57±1.08	5.42±1.06	5.17±1.34
Triglyceride (mmol/l)	2.26±1.21	2.17±1.20	2.76±1.34	1.79±0.84
Low density lipoprotein (mmol/l)	3.31±1.05	3.46±0.99	3.26±1.03	3.30±1.05
High density lipoprotein (mmol/l)	1.18±0.35	1.21±0.34	1.08±0.33	1.28±0.36
2 hr postprandial glucose (mmol/l)	7.46±3.09	10.0±3.19	7.53±3.19	6.34±2.21
Insulin (IU/l)			19.36±27.24	5.24±1.87
HOMA-IR			6.68±9.80	1.28±0.45
Glycated albumin (%)	13.01±4.22	15.75±4.70	12.68±4.71	12.15±2.79
hsCRP (mg/l)	6.70±17.66	3.14±5.11	10.78±24.01	3.97±11.6
Leptin(ng/ml)	19.40±11.59	18.19±11.62	25.08±9.18	14.12±11.27
Adiponectin(ug/ml)	12.86±6.18	12.38±6.30	11.01±5.67	14.94±6.08
Resistin(ng/ml)	33.77±15.15	30.96±16.13	36.05±17.87	32.59±11.12
Dialysis related indicators				
Total Kt/V	1.72±0.41	1.67±0.28	1.75±0.47	1.71±0.38
Residual kidney Kt/V	0.34±0.49	0.25±0.28	0.40±0.55	0.32±0.49
Total creatinine clearance (Ccr)	59.42±23.01	57.42±12.96	61.17±26.55	58.53±22.49
Renal Ccr	17.08±25.49	13.01±15.11	20.16±27.94	15.62±26.16
24-hr urine (ml)	455.80±630.68	526.25±674.16	484.92±692.26	400.76±552.16
Peritoneal creatinine clearance rate at 4 hr	0.62±0.11	0.61±0.11	0.63±0.10	0.62±0.11
Peritoneal glucose absorption at 4 hr	0.41±0.08	0.42±0.08	0.41±0.08	0.41±0.09
Peritoneal transport type				
High		2 (7.7%)	4 (6.1%)	3 (4.6%)
High-average		9 (34.6%)	21 (31.8%)	19 (29.2%)
Low- average		4 (15.4%)	6 (9.1%)	5 (7.7%)
Low		11 (42.3%)	35 (53.0%)	38 (58.5%)

Compared with the DM group: *means $P\leq0.05$; **means $P\leq0.01$; *** means $P\leq0.001$; Compared with the L group: # means $P\leq0.05$; ## means $P\leq0.01$; ### means $P\leq0.001$. WBC: white blood cell. hsCRP: high sensitive C-reactive protein

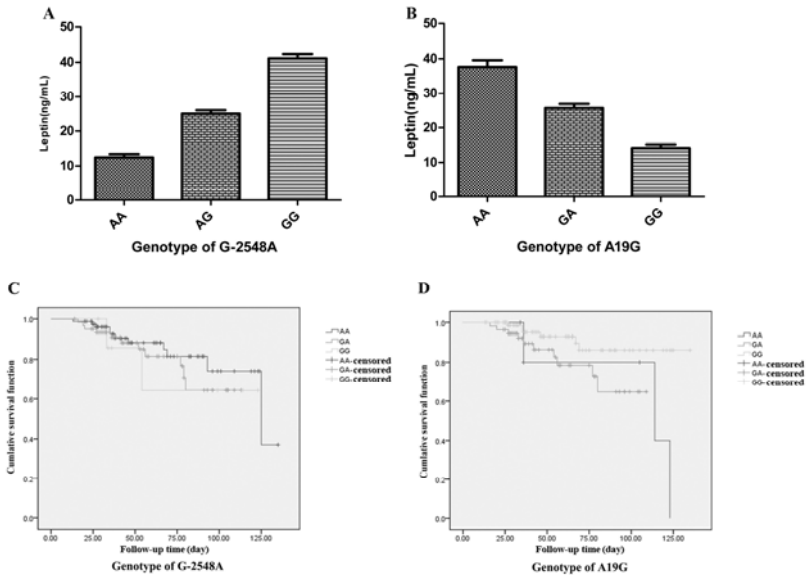

Figure 1. Serum leptin levels distribution (A and B) and Kaplan–Meier survival curves (C and D) for peritoneal dialysis (PD) patients with different genotypes. A and C: genotype of G-2548; B and D: genotype of A19G

Statistical analysis

Statistical analysis was produced in SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The two-sided $P<0.05$ were considered statistically significant for all tests.

Results

General information and dialysis related indicators of participants

The average age of total of 157 patients was 55±15 years old, and that of DM, H, and L groups was 61±13, 58±16, and 50±14 years old, respectively. The average of the age was higher in DM and H groups than L group ($P<0.001$). Totally 79 males were included in this study; 17 in DM group (n=26), 28 in H group (n=66), and 34 in L group (n=65). The difference in gender distribution among these groups was not significant ($P>0.05$). The BMI of L group was lower than that of DM and H groups ($P=0.007$). Height and mean arterial pressure of L group was higher than H group ($P<0.05$, $P<0.001$). The prevalence and the number of MS in DM and H groups were significantly higher than L group (all $P<0.001$). In addition, nPCR of L group was higher than DM group ($P=0.012$) and H group ($P=0.019$). There were no significant differences regarding SGA, dialysis age, calcium density, glucose absorption, and glucose concentration in the dialysate among those three groups (Table 1).

SNP genotyping profile

In this study, sequencing results of 157 patient samples showed that, five SNP loci (promoter regions -2548, -2558, and -2505, as well as exon regions -58 and -19) in two genomic regions were discovered. Genotypes of PD patients are shown in Table 2. Besides, the distribution of genotype frequencies among groups did complied with HWE, and it was capable for further studies.

Gene polymorphism and serum levels of leptin

According to the different genotypes, the selected patients were grouped and their serum leptin level were compared (Figure 1A and B). There were significant differences (GG was highest) between serum levels of leptin and genotypes of promoter polymorphism at locus -2548 ($P<0.001$). For the exon 19, serum leptin levels of GG were significantly different from the other two genotypes ($P=0.002$ for GA and $P<0.001$ for AA).

Table 2. Results of the single nucleotide polymorphisms of LEP in peritoneal dialysis (PD) patients

Locus	Homozygote (cases)1	Homozygote (cases) 2	Heterozygote (cases)
-2558	CC (156)	/	CT (1)
-2548	AA (82)	GA(66)	GG (9)
-2505	AA (154)	/	GA (3)
-58	GG (156)	GA (1)	/
19	GG (92)	GA (59)	AA(6)

Table 3. The relationship of -2548 polymorphism and clinical characteristics in peritoneal dialysis (PD) patients

	AA	GA	GG
Age (year)	53.50±14.59	57.61±16.49	53.78±9.00
Amount (male %)	51 (62.6)	26 (39.4)	2 (22.2)***
Height (cm)	164.54±7.98	160.92±7.82##	160.44±7.43
Body mass index (kg/m2)	22.18±3.05	24.21±3.71###	26.53±4.21###
Systolic blood pressure (mmHg)	100.33±16.35	101.72±13.32	95.11±14.04
Subjective global assessment			
Normal (%)	55 (67.1)	52 (78.8)	6 (66.7)
Moderate malnutrition (%)	25 (30.5)	13 (19.7)	2 (22.2)
Severe malnutrition (%)	2 (2.4)	1 (1.5)	1 (11.1)
Dialysis age (month)	37.02±30.43	34.95±26.94	35.56±33.02
Normalized protein catabolic rate	0.87±0.17	0.79±0.21#	0.77±0.21
Metabolic syndrome (%)	34 (41.5)	36 (54.5)	6 (66.7)
Number of factors	2.23±0.95	2.62±1.00#	3.00±0.87#
Dialysate volume (L/day)	7.24±1.28	7.24±1.30	7.36±0.88
Dialysate calcium concentration			
1.25(%)	42 (51.2)	29 (43.9)	4 (44.4)
1.75(%)	40 (48.8)	37 (56.1)	5 (55.6)
Glucose intake (g/day)	64.57±18.07	65.08±17.05	70.11±19.05
Glucose absorption (g/day)	38.21±12.12	38.03±12.02	42.51±15.83
Laboratory index			
WBC（×109/l）	7.70±2.64	8.29±3.12	8.82±1.67
Hemoglobin(g/l)	110.45±20.91	112.35±17.66	114.00±18.63
Calcium (mmol/l)	2.29±0.22	2.29±18	2.44±0.37#
Phosphorus (mmol/l)	1.57±0.44	1.59±0.46	1.49±0.49
Parathyroid hormone (pg/ml)	446.96±371.21	348.90±378.37	357.82±391.60
Albumin (g/l)	37.51±4.85	38.28±4.32	37.63±6.22
Cholesterol (mmol/l)	5.15±1.26	5.47±1.08	6.14±0.86#
Triglyceride (mmol/l)	2.16±1.20	2.35±1.22	2.58±1.27
Low density lipoprotein (mmol/l)	3.15±1.09	3.44±1.02	3.84±0.55
High density lipoprotein (mmol/l)	1.19±0.37	1.17±0.33	1.26±0.42
2h postprandial glucose (mmol/l)	7.32±3.23	7.34±2.55	9.62±4.64#
HOMA-IR	3.08±4.05	6.66±11.31##	6.51±4.31
Glycated albumin (%)	13.13±4.19	12.91±4.35	12.69±4.11
hsCRP (mg/l)	6.27±12.82	6.25±22.53	13.87±15.19
Leptin (ng/ml)	12.46±8.70	25.05±8.24	41.13±3.79
Adiponectin(ug/ml)	13.23±6.76	12.68±5.50	10.81±5.44
Resistin (ng/ml)	34.06±15.82	30.79±11.99	53.10±16.80###
Dialysis related indicators			
Total Kt/V	1.66±0.32	1.71±0.38	1.80±0.29
Residual kidney Kt/V	0.29±0.41	0.33±0.42	0.46±0.53
Total creatinine clearance (Ccr)	57.12±18.02	56.72±17.13	61.60±16.68
Renal Ccr	15.00±21.80	14.60±19.57	21.18±22.49
24-hr urine (ml)	435.54±596.74	528.75±696.13	334.58±147.22
Peritoneal creatinine clearance rate at 4 hr	0.63±0.11	0.62±0.10	0.62±0.13
Peritoneal glucose absorption at 4 hr	0.41±0.08	0.42±0.08	0.40±0.10
Peritoneal transport type			
High	4 (4.9)	4 (6.1)	1 (11.1)
High-average	27 (32.9)	19 (28.8)	3 (33.3)
Low- average	6 (7.3)	8 (12.1)	1 (11.1)
Low	45 (54.9)	35 (53.0)	4 (44.4)

Comparison between the three groups: *means $P \leq 0.05$; **means $P \leq 0.01$; *** means $P \leq 0.001$; Compared with the AA group: # means $P \leq 0.05$; ## means $P \leq 0.01$; ### means $P \leq 0.001$. hsCRP: high sensitive C-reactive protein.

Table 4. The relationship of 19 polymorphism and clinical characteristics in peritoneal dialysis (PD) patients

	AA	GA	GG
Age (year)	60.91±8.97	57.42±16.47	53.00±14.67
Amount (male %)	2 (33.3)	25 (42.4)	52 (56.5)
Height (cm)	162.91±6.25	161.33±8.10	163.78±8.14
Body mass index (kg/m2)	25.20±3.57	24.08±3.93	22.48±3.19#⊿⊿
Systolic blood pressure (mmHg)	97.21±13.56	100.73±12.39	100.98±16.83
Subjective global assessment			
Normal (%)	3 (50)	46 (78.0)	64 (69.6)
Moderate malnutrition (%)	3 (50)	11 (18.6)	26 (28.3)
Severe malnutrition (%)	0 (0)	2 (3.4)	2 (2.2)
Dialysis age (month)	38.91±31.97	34.75±25.11	36.63±31.33
Normalized protein catabolic rate	0.74±0.13	0.79±0.17	0.87±0.19#⊿⊿
Metabolic syndrome (%)	5 (83.3)	29 (49.2)	42 (45.7)
Number of factors	3.00±0.78	2.55±0.98	2.29±0.98#
Dialysate volume (l/day)	7.64±0.81	7.36±1.13	7.14±1.39
Dialysate calcium concentration			
1.25 (%)	3 (50)	26 (44.1)	46 (50)
1.75 (%)	3 (50)	33 (55.9)	46 (50)
Glucose intake (g/day)	68.45±15.98	66.48±16.03	63.71±18.96
Glucose absorption (g/day)	40.69±12.12	39.36±11.82	37.40±12.81
Laboratory index			
WBC (×109/l)	7.59±2.39	8.50±3.17	7.71±2.58
hemoglobin (g/l)	119.60±14.71	111.32±18.33	110.57±20.52
Calcium (mmol/l)	2.22±0.28	2.31±0.18	2.30±0.23
Phosphorus (mmol/l)	1.31±0.27	1.60±0.45#	1.59±0.46
Parathyroid hormone (pg/ml)	257.55±252.80	360.48±399.75	446.95±367.66
Albumin (g/l)	33.71±5.31	38.12±4.18##	38.18±4.77##
Cholesterol (mmol/l)	5.48±1.06	5.47±1.05	5.23±1.30
Triglyceride (mmol/l)	2.04±0.80	2.36±1.20	2.22±1.27
Low density lipoprotein (mmol/l)	3.53±0.97	3.43±0.95	3.20±1.12
High density lipoprotein (mmol/l)	1.16±0.30	1.15±0.35	1.21±0.36
2h postprandial glucose (mmol/l)	10.68±3.81	7.33±2.62###	7.13±3.09###
HOMA-IR	4.47±3.71	6.86±11.83	3.37±4.16⊿⊿
Glycated albumin (%)	14.13±3.63	13.18±4.47	12.74±4.13
hsCRP (mg/l)	9.01±14.46	7.78±23.86	5.65±12.23
Leptin(ng/ml)	24.79±16.11	25.73±9.03	14.29±10.05##⊿⊿⊿
Adiponectin (ug/ml)	11.21±6.90	12.22±5.34	13.51±6.60
Resistin(ng/ml)	31.19±16.54	32.48±14.81	35.01±15.28
Dialysis related indicators			
Total Kt/V	1.68±0.31	1.69±0.35	1.70±0.35
Residual kidney Kt/V	0.35±0.44	0.30±0.41	0.32±0.43
Total creatinine clearance (Ccr)	60.26±16.35	55.18±15.33	58.24±19.05
Renal Ccr	18.15±22.78	12.69±18.39	16.57±22.23
24-hr urine (ml)	373.18±705.06	458.93±636.62	464.85±623.77
Peritoneal creatinine clearance rate at 4 hr	0.65±0.09	0.62±0.10	0.62±0.11
Peritoneal glucose absorption at 4 hr	0.41±0.46	0.41±0.08	0.42±0.09
Peritoneal transport type			
High	1 (16.7)	3 (5.1)	5 (5.4)
High-average	2 (33.3)	18 (30.5)	29 (31.5)
Low- average	3 (50.0)	6 (10.2)	9 (9.8)
Low	0 (0)	32 (54.2)	49 (53.3)

Comparison between the three groups: *means $P\leq0.05$; **means $P\leq0.01$; *** means $P\leq0.001$; Compared with the AA group: # means $P\leq0.05$; ## means $P\leq0.01$; ### means $P\leq0.001$; Compared with the GA group: ⊿means $P\leq0.05$; ⊿⊿means $P\leq0.01$; ⊿⊿⊿means $P\leq0.001$. hsCRP: high sensitive C-reactive protein

Laboratory tests and gene polymorphism at position -2548 in the promoter region

BMI of AA, GA and GG groups were different ($P<0.001$). The number of factors for MS in AA group was significantly less than GA group ($P=0.024$) or GG group ($P=0.015$). Additionally, male ratio of the GG group was lower than AA and GA groups ($P<0.001$) (Table 3).

HOMA-IR and nPCR of GA group were obviously higher, while, body length of GA group was significantly lower than AA group. 2 hr postprandial glucose, cholesterol, and calcium of the GG group were significantly higher than the AA group ($P<0.001$, $P<0.05$, and $P<0.05$, respectively) (Table 2).

Laboratory tests and gene polymorphism at position 19 in the exon region

As shown in Table 4, BMI of both AA and GA groups were significantly higher than the GG group

Table 5. Leptin G-2548A and A19G genotype and frequencies in each group of peritoneal dialysis patients

Polymorphisms		Group		
		DM (N=26)	IR>2.05(N=66)	IR>2.05(N=65)
G-2548A Genotype	AA	9	30	43
	GA	15	31	20
	GG	2	5	2
Allele	A (%)	33	91	106
	G (%)	19	41	24
A19G				
Genotype	AA	1	4	1
	GA	13	28	18
	GG	12	34	46
Allele	A (%)	15	36	20
	G (%)	37	96	110

G-2548A: χ2 of Genotype =7.4868, *P*=0.02; χ2 of Allele=8.352, *P*=0.015; A19G: χ2 of Genotype =7.231, *P*=0.027; χ2 of Allele=6.653, *P*=0.036

(*P*=0.008, *P*=0.017). Serum albumin (SA) of the AA group was lower than GA and GG groups (*P*=0.004, *P*=0.003). 2h postprandial plasma glucose of AA was significantly higher than GA (*P*=0.001) and GG (*P* <0.001) groups.

Blood phosphorus of AA group was lower than GA group (*P*=0.049). nPCR of AA group was lower than GG group (*P*=0.022), and the number of factors for MS in AA group was also higher than the GG group (*P*=0.024). HOMA-IR of GA group was higher than the GG group (*P*=0.01).

Leptin gene polymorphisms and prognosis of patients

Survival analysis found no mortality differences among the three groups (GG, GA, and AA) at position -2548 in the promoter region druing follow-up (*P*=0.41) (Figure 1C). Survival curves of GG, GA, and AA groups at position 19 in the exon region showed significant difference (*P*=0.032); especially in the AA group which was lowest (Figure 1D).

Promoter and exon gene polymorphisms of patients with diabetes, high IR, and low IR

Gene polymorphism at position -2548 in the promoter region of DM, H, and L groups was different (χ2 =7.487, *P*=0.02). Alleles among these three groups were also different (χ2 =8.352, *P*=0.015) (Table 5).

Gene polymorphism at position 19 in the exon region of DM, H, and L groups was different (χ2 =7.231, *P*=0.027). Alleles among these three groups were also different (χ2 =6.653, *P*=0.036) (Table 5).

No statistically significant difference was detected in genotype distribution of other gene loci including -2505, -2558, -58 of PD patients.

Discussion

Leptin is generated by the LEP encoding gene (Obese, Ob) (17). In extremely obese patients, LEP gene is highly expressed in subcutaneous fat and intraabdominal fat (18). It was first discovered in ob/ob mice with a mutation in codon 105 which resulted in decreased leptin secretion (19). This mutation causes obesity, hyperphagia, loss of weight, even IR, and infertility. Leptin supplement can make it return to a normal state. There are almost 84% homology of human and mouse gene sequence of LEP gene. However, in humans, there is no similar LEP mutation with mouse (20). Therefore, other mutations in humans may be analogous for these functions. Our study in 157 PD patients found five gene polymorphism sites (the promoter regions of -2548, -2558, -2505, and exon regions of -58 and -19) in two gene regions. Among them, promoter region -2548 and exon -19 polymorphism showed correlation with serum leptin value.

In our study, the GG genotype of -2548 region had higher BMI and leptin than that of GA, especially AA, which was in line with other studies. Mammes *et al* (9) found that in the general population, the -2548 G allele was more common in obese population (BMI >27 kg/m^2) than normal (BMI <27 kg/m^2). Leptin G-2548A promoter polymorphism was also associated with increased plasma leptin level (21).

Meanwhile, at G-2548A promoter position 2 hr postprandial glucose, cholesterol, HOMA-IR value of AA genotype were lower, and the nPCR was higher than two other genotypes in our PD patients. Currently, there was no reference showedLEP gene polymorphism may associated with glucolipid metabolism in PD patients. Our finding may indicated AA genotype group has lower BMI and leptin , AA genotype may have certain protective effects including the lower degree of glucolipid metabolic disorders and the lower incidence of MS in PD patients However, G-2548A gene polymorphism may have no effect on patients' prognosis.

In addition, we also found at position 19 in exon that BMI of AA genotype was higher than GA especially than GG genotype. Leptin level of AA genotype was similar with GAA and was higher than GG. These results demonstrated that A allele frequencies of LEP gene polymorphism at position 19 in exon was positively correlated with serum leptin as well as BMI, which was similar with Hager *et al* (6). A19G LEP polymorphism was also correlated with the change in fasting insulin (8). In particular, compared with two other genotypes, 2 hr postprandial glucose of AA genotype was the highest, and the serum albumin was the lowest. We considered that changes of these two indicators may be secondary processes: the increased leptin level induced the generation of IR, then the incidence of MS was increased, and so the disorders of glucose and lipid metabolism appeared, which finally led to worse nutritional status. The decreased nPCR and serum phosphorus values of AA genotype in the study also indicated the poor nutritional intake. Moreover, the Kaplan-Meier survival analysis showed that leptin A19G polymorphism, as a candidate SNP for several cancers and hypertension (5, 22), may be a prognostic risk factor for patients with poor prognosis. In our patient the poor survival rate may also relate to the low serum albumin levels in the AA genotype group.

Grouping comparison of -2548 and A19G genotype found that in DM and IR (H) groups, gene frequency of -2548 G allele in the promoter region and 19 A allele in exon was increased. This results may suggested that these two LEP gene polymorphisms have affected the leptin serum concentrations and also have participated in the IR, hyperleptinemia, and other pathophysiologic processes which may also explain some of the causes of poor prognosis in type 2 diabetic patients treated with PD. By contrast, patients with significantly increased frequencies of -2548 locus A allele and 19 G allele polymorphism had a maintained low level of serum leptin, which could tolerate a lot of intraperitoneal absorption of glucose, performed with the decreased incidence of IR, and then have a protective effect on patients.

Conclusion

-2548 in the promoter region and 19 in the exon region polymorphisms were correlated with serum leptin values and BMI in PD patients. Leptin A19G polymorphism may be an unfavorable prognostic factor in PD patients. Genetic polymorphisms may also participate in pathophysiologic process including IR, hyperleptin-emia, glucose metabolism disorders of PD patients. These results may providethe genetic polymorphism may help the early diagnosis of complications in PD patients. Undoubtedly, more experiments with larger sample size are still needed to validate these results.

Acknowledgment

This study was supported in part by the National Basic Research Program of China 973 Program (No. 2012CB517600, No. 2012CB517602); the study was also sponsored by the National Natural Science Foundation of China (81370794, 81102700, 81373865). The results described in this paper were part of student thesis.

Conflict of interest

All authors declare that they have no conflict of interest.

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