CLINICAL STUDY

Association of leptin receptor polymorphism with insulin resistance

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Abstract

Objective: Although the role of the leptin receptor (LEPR) in obesity is well recognized, its role in insulin sensitivity remains unknown.

Design: This was a cross-sectional study to investigate the impact of the Q223R polymorphism of the LEPR gene on insulin sensitivity.

Methods: Sixty-seven glucose-tolerant Caucasians were enrolled. The insulin sensitivity index and glucose clearance were measured using a hyperglycemic clamp. Genomic DNA was extracted for genotyping.

Results: We identified 20 QQ, 31 QR, and 16 RR subjects. The three genotypic groups were similar in age, gender distribution, body mass index, waist–hip ratio, and blood pressure, but differed in the insulin sensitivity index ($P = 0.0180$) and in glucose clearance ($P = 0.0220$). Multivariate analyses showed that the Q223R polymorphism was independently associated with the insulin sensitivity index ($P = 0.0317$) and glucose clearance ($P = 0.0436$). A trend of increasing low density lipoprotein cholesterol concentration was also observed ($P = 0.0447$).

Conclusion: We have reported the first association of the Q223R polymorphism of the LEPR gene with insulin resistance.

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Introduction

The leptin receptor (LEPR) is a member of the cytokine receptor family, which plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure. The leptin receptor is located on chromosome 1p31, which has been linked to an acute insulin response in Pima Indians (1). However, no mutations within this gene were found to be responsible for this linkage in Pima Indians (2). Although the linkage failed to be replicated in the same population (3), this region is also linked to diabetes and post-challenge plasma glucose concentrations in an Old Order Amish population (4) and also to the 24-h respiratory quotient in Pima Indians (5). The latter two linkage studies suggested a link between this locus and insulin resistance.

Mutated LEPR plays a crucial role in the pathogenesis of obesity and/or diabetes in *db/db* mice, Zucker fatty (*fa/fa*) rats, and Koletsky rats. Although it does not play a major role in human diseases, markers at this locus have been demonstrated to be associated with obesity/diabetes phenotypes in the human (4, 5). Among them, the Q223R polymorphism has been shown to affect receptor function (6). However, the relationship of this polymorphism with insulin sensitivity has not

been examined as yet. In this study, we examined the impact of this polymorphism on insulin sensitivity in a group of well-characterized subjects.

Subjects and methods

The study complied with the recommendations of the Declaration of Helsinki and was approved by the Institutional Review Board. Written informed consent was obtained from each participant at entry to the study. Sixty-seven healthy Caucasian subjects (36 females and 31 males) were recruited from West Los Angeles through an advertisement on the campus of the University of California, Los Angeles. The detailed study protocol has been described previously (7). The demographic features of the study subjects are shown in Table 1. They had normal glucose tolerance and were normotensive at entry to the study and then underwent a hyperglycemic clamp as described previously (8). Briefly, after an overnight fast, participants received a bolus of 50% dextrose solution (11.4 gm/m^2) at $t = 0 \text{ min}$. Continuous infusion of 30% dextrose solution was commenced at $t = 15 \text{ min}$ at a variable rate which was adjusted every 5 min so as to maintain a plasma glucose concentration around 10 mmol/l toward $t = 180 \text{ min}$. The insulin

Table 1 Clinical characteristics of the 67 study subjects, 36 (54%) of whom were female.

	Means $\pm$ S.D.	Minimum	Maximum
Age (years)	27 $\pm$ 6	19	39
Body mass index (kg/m ²)	24.17 $\pm$ 3.95	17.58	34.26
Waist–hip ratio (cm/cm)	0.804 $\pm$ 0.077	0.649	1.033
Systolic blood pressure (mmHg)	116 $\pm$ 11	94	137
Diastolic blood pressure (mmHg)	67 $\pm$ 8	48	89
Steady-state plasma glucose (mmol/l)	10.06 $\pm$ 0.35	9.32	10.77
First-phase insulin response (pmol/l)	1597 $\pm$ 917	571	4569
Second-phase insulin response (pmol/l)	389 $\pm$ 204	104	1567
Insulin sensitivity index (μ m/m ² per min per pmol)	7.447 $\pm$ 3.461	1.728	16 532
Glucose clearance (l/m ² per min per pmol)	0.750 $\pm$ 0.364	0.171	1.774

sensitivity index was calculated by dividing the average glucose infusion rate during the last 60 min of the clamp by the average plasma insulin level. To adjust for the mass effect of glucose during the hyperglycemic clamp, glucose clearance was defined as the insulin sensitivity index divided by the plasma glucose concentration. The coefficient of variation for steady-state plasma glucose levels was $5.8 \pm 2.4\%$. The first-phase insulin response was the sum of plasma insulin levels during the first 10 min ($t = 2.5, 5, 7.5$, and 10 min) and the second-phase insulin response was the average of plasma insulin levels at $t = 130, 140, 150, 160, 170$, and 180 min. Although the gold standard for the measurement of insulin sensitivity is the euglycemic clamp, the hyperglycemic clamp provides both insulin sensitivity and β -cell function from a single procedure. Furthermore, insulin sensitivity measured by the hyperglycemic clamp has an excellent correlation with the insulin sensitivity measured by the euglycemic clamp (9–11). We therefore chose the hyperglycemic clamp for this study, which allowed us to assess insulin sensitivity and β -cell function. Genomic DNA was isolated from peripheral lymphocytes (7). Primer sequences were determined and polymerase chain reactions were performed as described previously (6).

Skewed variables were logarithmically transformed to normality before analysis. These variables were: body mass index, waist–hip ratio, plasma insulin concentrations, first-phase insulin response, second-phase

insulin response, insulin sensitivity index, and glucose clearance. Differences in continuous variables between groups of subjects were tested with ANOVA. Differences in proportions were evaluated by a chi-square test or Fisher exact test, as appropriate. To examine the influence of multiple variables on the insulin sensitivity index or glucose clearance, a stepwise regression analysis using general linear models was performed with a backward stepwise option as described previously (7). A nominal P value of less than 0.05 was considered significant. Data are presented as arithmetic means $\pm$ S.D. unless otherwise specified. SYSTAT 10.0 for Windows from SPSS Inc. (Chicago, IL, USA) was used for statistical analysis.

Results

This study included 67 glucose-tolerant and normotensive subjects, confirmed by a standard 75 g oral glucose tolerant test with blood pressure $< 140/90$ mmHg. We identified 20 QQ, 31 QR, and 16 RR subjects with an allelic frequency of 0.53 for the Q allele and 0.47 for the R allele. The observed genotypic frequency was in compliance with the Hardy–Weinberg equilibrium ($P = 0.94$). Among the three genotypic groups, there were no differences in age, gender distribution, body mass index, waist–hip ratio, or systolic and diastolic blood pressure (Table 2). The fasting lipid profile was

Table 2 Clinical characteristics by the Q223R polymorphism; mean with 95% confidence intervals in parentheses; ^ageometric mean with 95% confidence intervals in parentheses.

	QQ ($n = 20$)	QR ($n = 31$)	RR ($n = 16$)
Age (years)	27 (24, 30)	27 (25, 30)	26 (24, 29)
Females (%)	11 (55%)	16 (52%)	9 (56%)
Body mass index ^a (kg/m ²)	23.98 (22.34, 25.74)	23.96 (22.59, 25.43)	24.60 (22.62, 26.76)
Waist–hip ratio ^a (cm/cm)	0.790 (0.755, 0.827)	0.799 (0.772, 1.209)	0.812 (0.770, 0.857)
Systolic blood pressure (mmHg)	113 (108, 117)	117 (114, 121)	116 (109, 122)
Diastolic blood pressure (mmHg)	65 (61, 69)	69 (66, 72)	68 (63, 73)
Total cholesterol ^b (mg/dl)	144 (134, 155)	165 (156, 175)	158 (141, 176)
HDL cholesterol (mg/dl)	47 (42, 53)	52 (47, 56)	45 (40, 51)
LDL cholesterol ^c (mg/dl)	81 (70, 91)	97 (88, 105)	99 (83, 115)
Triglycerides (mg/dl)	83 (63, 102)	84 (68, 101)	74 (57, 92)

HDL, high density lipoprotein.

^b $P = 0.0287$; ^c $P = 0.0447$.

notably different in total cholesterol ($P = 0.0287$) and low density lipoprotein (LDL) cholesterol ($P = 0.0447$; Table 2). During the oral glucose tolerance test, differences were noted in the post-challenge plasma glucose concentration at 60 min ($P = 0.0212$) and plasma insulin concentration at 60 min ($P = 0.0372$; Table 3). The plasma glucose concentration at 90 min was marginally different ($P = 0.0599$).

Insulin sensitivity and β -cell function were assessed by a hyperglycemic clamp. The steady-state plasma glucose concentrations were almost identical among the three groups, while differences were observed in the insulin sensitivity index ($P = 0.0180$) and glucose clearance ($P = 0.0220$; Table 3). Since various covariates could potentially affect the insulin sensitivity, multivariate analysis was conducted by considering the influence of age, gender, body mass index, waist-hip ratio, systolic blood pressure, diastolic blood pressure, and Q223R polymorphism (Table 4). The Q223R polymorphism along with the body mass index, diastolic blood pressure, age, and gender explained 39.59% and 39.40% of the variances in the insulin sensitivity index and glucose clearance respectively.

Discussion

Although the role of Q223R polymorphism at the leptin receptor gene has been examined in various studies, its impact on insulin sensitivity and the metabolic syndrome has not been investigated previously. The association of this polymorphism with insulin resistance was demonstrated in the univariate analysis and confirmed in the multivariate analysis. This polymorphism is an independent factor affecting 6–7% of insulin sensitivity. This association is in agreement with the fact that the subjects with the R allele are at risk for the

metabolic syndrome, in which insulin resistance plays a major role. In contrast to the subjects without the R allele, the subjects with at least one R allele had insulin-resistant phenotypes, higher total and LDL cholesterol ($P = 0.0287$ and $P = 0.0447$), higher plasma glucose concentration at 60 and 120 min ($P = 0.0212$ and $P = 0.0599$), and higher plasma insulin concentration at 60 min ($P = 0.0373$). The R allele is therefore a risk factor for insulin resistance.

Although meta-analyses of the published reports conclude that there was no interaction of the Q223R polymorphism with obesity and its traits (12, 13), several reports showed the association of this polymorphism with various traits in obesity and metabolism (14–19). The latter association suggests a possible association between this polymorphism and insulin resistance as is demonstrated in this study. However, not all the studies are in agreement with the association of the R allele with obesity phenotypes. Interestingly, just like our population in this study, most of the positive associations of the R allele with obese or insulin-resistant phenotypes were observed in young (mean age < 30 years old) and healthy populations (14, 16, 19). In contrast, negative associations were found in postmenopausal women (17, 18) or in pathological states, such as impaired glucose tolerance (17) or overfeeding (15). It is possible therefore that this polymorphism contributes to the initiation of the events leading to insulin resistance in a subset of subjects. Additional studies are required to resolve this issue.

LEPR is a single transmembrane domain receptor with two cytokine domains, which binds to leptin (20). In Zucker fatty (*fa/fa*) rats, a missense mutation (an A to C conversion at nucleotide position 806) in the extracellular domain of the LEPR gene results in a single amino acid change from Q to P at position 269 (21). The *fa* mutation gives rise to a defective LEPR

Table 3 Oral glucose tolerance tests and hyperglycemic clamps by the Q223R polymorphism; mean with 95% confidence intervals in parentheses; ^ageometric mean with 95% confidence interval in parentheses.

	QQ ($n = 20$)	QR ($n = 31$)	RR ($n = 16$)
Oral glucose tolerance test			
Fasting plasma glucose (mmol/l)	4.58 (4.41, 4.76)	4.71 (4.59, 4.84)	4.69 (4.51, 4.88)
Plasma glucose at 30 min (mmol/l)	7.27 (6.71, 7.83)	7.62 (7.14, 8.11)	6.99 (6.32, 7.66)
Plasma glucose at 60 min ^b (mmol/l)	6.30 (5.80, 6.80)	7.38 (6.83, 7.92)	6.75 (6.05, 7.46)
Plasma glucose at 90 min (mmol/l)	5.66 (5.12, 6.21)	6.17 (5.67, 6.67)	6.00 (5.24, 6.76)
Plasma glucose at 120 min (mmol/l)	5.06 (4.53, 5.60)	5.77 (5.43, 6.11)	5.69 (5.06, 6.31)
Fasting plasma insulin ^a (pmol/l)	55 (48, 64)	58 (52, 65)	64 (56, 74)
Plasma insulin at 30 min ^a (pmol/l)	289 (229, 364)	354 (302, 414)	365 (268, 497)
Plasma insulin at 60 min ^{a,c} (pmol/l)	248 (195, 317)	345 (297, 401)	333 (263, 420)
Plasma insulin at 90 min ^a (pmol/l)	204 (163, 255)	229 (187, 280)	254 (179, 362)
Plasma insulin at 120 min ^a (pmol/l)	165 (125, 218)	200 (163, 246)	226 (169, 298)
Hyperglycemic clamp			
Steady-state plasma glucose (mmol/l)	10.06 (9.94, 10.18)	10.03 (9.89, 10.18)	10.12 (9.91, 10.32)
First-phase insulin response ^a (pmol/l)	1264 (1014, 1576)	1470 (1185, 1825)	1415 (1135, 1765)
Second-phase insulin response ^a (pmol/l)	330 (262, 415)	344 (297, 399)	393 (302, 513)
Insulin sensitivity index ^{a,d} ($\mu\text{m}/\text{m}^2$ per min per pmol)	8.436 (7.030, 10.122)	6.374 (5.392, 7.536)	5.491 (4.155, 7.258)
Glucose clearance ^{a,e} (l/m^2 per min per pmol)	0.839 (0.700, 1.006)	0.645 (0.539, 0.772)	0.543 (0.414, 0.713)

^b $P = 0.0212$; ^c $P = 0.0372$; ^d $P = 0.0180$; ^e $P = 0.0220$.

Table 4 Multivariate analysis of the Q223R polymorphism on the insulin sensitivity index and glucose clearance.

Dependent variable	Co-variate entered	Partial r^2	P
Insulin sensitivity index	Body mass index	0.1265	0.0007
	Diastolic blood pressure	0.0766	0.0077
	Age	0.0600	0.0176
	Q223R polymorphism	0.0737	0.0317
	Gender	0.0464	0.0358
Glucose clearance	Body mass index	0.1045	0.0021
	Diastolic blood pressure	0.0982	0.0028
	Age	0.0618	0.0162
	Q223R polymorphism	0.0667	0.0436
	Gender	0.0536	0.0247

that is unable to constitutively activate the signal pathway and is also highly impaired for ligand-induced activation (22). Since both Q223R polymorphism and the *fa* mutation occur in the first cytokine domain, it is highly possible that the Q223R polymorphism could also affect the function of LEPR. Furthermore, the conservation of this amino acid among the human, the rat, and the mouse suggests the possibility of a functional importance of this amino acid. A carefully designed study is required to examine the functional consequence of this polymorphism.

Insulin resistance plays a key role in the pathogenesis of type 2 diabetes. Although genetic influence is well recognized in type 2 diabetes, less than 50% of insulin sensitivity is inherited (23). Since type 2 diabetes is a multifactorial disease, each genetic factor will only have a very modest impact. We have observed that the Q223R polymorphism accounts for 6–7% of the variance in insulin sensitivity. Furthermore, this polymorphism has been shown to explain only 3–5% of body mass index and percent fat mass in a young Greek population (19). These observations indicate a very modest impact by this polymorphism.

In conclusion, we have present the first study of the Q223R polymorphism on insulin sensitivity. In a healthy and glucose-tolerant Caucasian population, this polymorphism is a modest but independent determinant of insulin sensitivity. The subjects with the R allele are at risk for the metabolic syndrome. However, this association does not imply a causal relationship between the Q223R polymorphism and insulin resistance. Nevertheless, the association suggests that the LEPR could play a role in the pathogenesis of insulin resistance. Confirmation studies in this ethnic group as well as other ethnic groups are required.

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