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ORIGINAL CONTRIBUTION

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β_2-Adrenergic receptor mutation and abdominal obesity risk: Effect modification by gender and HDL-cholesterol

Summary *Objective and design* A case-control study was conducted to examine the association between the 27Glu polymorphism of the β_2-adrenergic receptor gene (ADRB2) and the risk of abdominal obesity (defined by a waist/hip ratio: WHR higher than 0.85). *Methods* The case series encompassed 112 obese subjects with body mass index (BMI) > 30 kg/m^2 and WHR > 0.85 and no other major disease except for type 2 diabetes, while the controls were 127 healthy subjects, BMI < 25 kg/m^2 and WHR < 0.85. *Results* The association between the risk of abdominal obesity and the 27Glu polymorphism was estimated using multivariate logistic regression. A higher crude odds ratio (OR) of 4.08 (95 % confidence interval: 0.98–16.3) for the 27Glu allele was found among men, while no increased risk was apparent among female participants. Moreover, when the model was adjusted for age, male subjects carriers of the 27Glu allele had a significant tenfold higher risk of abdominal obesity (OR = 10.31; 95 % CI: 1.4–76.8) and the product-term for the interaction (effect modification) between gender and the ADRB2 mutation was near to the limits of statistical significance (Likelihood ratio test p = 0.056). Interestingly, we also found an effect modification with higher OR among individuals with low HDL-cholesterol (< 1.5 mmol/l) after adjustment for age and gender (OR = 2.87 95 % CI 1.09–7.50) and the product-term for interaction between the 27Glu allele and HDL-cholesterol was statistically significant (Likelihood ratio test p = 0.003). *Conclusions.* Our results showed that the 27Glu allele of the ADRB2 gene appears to be a risk factor for abdominal obesity among male subjects, specially among those with lower HDL-cholesterol levels.

Key words polymorphism – abdominal obesity risk – HDL-cholesterol – gender – 27 Glu allele of the ADRB2

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Introduction

It is well established that obesity is under strong genetic influences, with up to 40 % of the variation in body fat content being attributed to genetic factors [1]. Genes that are involved in the regulation of catecholamine functions may be of particular importance for human obesity. Thus, the β_2-adrenergic receptor gene (ADRB2) is a major lipolytic receptor in human fat cells [2]. Two common polymorphisms of the ADRB2 gene, characterized by an amino acid replacement of arginine by glycine in codon 16 (Arg16Gly) and glutamine by glutamic acid in codon 27 (Gln27Glu) have been explored in several diseases such as hypertension and obesity [3–4]. A relationship between the Arg16Gly polymorphism and an altered function of the ADBR2 has been reported, thus leading to a decreased agonist sensitivity [5]. Meanwhile, the Gln27Glu variant was also found to be linked to obesity in some populations [6–9]. In men, the 27Glu allele has been associated with increased BMI and subcutaneous fat [10] and with elevated leptin and

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triglycerides levels [3, 11], while in women, the 27Glu variant was reported to be linked to increased BMI, body fat mass and waist to hip ratio [5]. However, other studies in Caucasians (Danish men, Austrian women and German subjects); however, found no association between the Gln27Glu variant of the ADRB2 gene and obesity [12–14]. Since the effects of the 27Glu allele on the development of obesity are still controversial, we conducted a case-control study in a Spanish population to specifically evaluate the association between the 27Glu polymorphism of the ADRB2 and the risk of abdominal obesity.

Methods

Study population

The study population, recruited from the Endocrinology and Occupational Health Departments at the Navarra Hospital between January 1999 and June 2000, comprised 239 Spanish subjects, aged 20–60 years. We based the study on a case-control design, defining cases of abdominal obesity as those individuals having both BMI >30 kg/m^2 and a WHR >0.85. Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrine diseases or serious intercurrent illness. Subjects with type 2 diabetes, not receiving glucose-lowering agents, were eligible as cases (9%). Controls were healthy subjects having a BMI <25 kg/m^2 and WHR <0.85 with no apparent disease and blood pressure below 120/90. In total, 112 obese patients (BMI mean: 37.7 Standard deviation (SD: 5.3 kg/m^2) and 127 normal weight subjects (BMI mean: 22.0; SD: 1.8 kg/m^2) were selected. Response rates were acceptable (65% for cases and 75% for controls) and the interviews were all conducted in a medical environment with little or no time pressure.

The study was approved by the Ethics Committee of the University of Navarra and all subjects provided written informed consent for participation. The reported investigation has been carried out according to the principles of the Declaration of Helsinki II.

Procedures

Anthropometric measurements were made by standard procedures and fat mass was measured by bioelectrical impedance [15]. Weight, height and the waist to hip ratio (WHR) were measured by conventional protocols. Following a 12 h fast, venous blood samples were obtained and serum glucose and lipids were measured by enzymatic methods. Serum insulin was measured by radioimmunoassay (DP Corporation) and plasma leptin by enzymeimmunoassay (Linco).

Blood samples were taken for the extraction and characterization of genomic DNA from leukocytes as previously described [4, 5]. The DNA segment containing codon 27 of the ADRB2 gene was amplified by polymerase chain reaction (PCR) carried out in a volume of 30 µl containing 200 ng of genomic DNA; 10 pmol of each primer (upstream: 5'-ccgccgtgggtccgcc-3'; downstream: 5'-ccatgaccagatcagcac-3'), 200 µM of dNTP; 1.5 mM magnesium chloride; 3 µl reaction buffer (10X: 160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8 at 25 °C) and 0.1% Tween–20) and 0.8 unit of *Taq* polymerase (BIOTAQ™, Bioline). The PCR reactions began with denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 65 °C for 30 seconds and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 10 minutes. Ten microliters of PCR products (310 bp) were digested with the addition of 10 µl of a mixture containing 6 units of *ItaI*, a restriction enzyme specific for the sequence GC/NGC (Roche Diagnostics) and reaction buffer. This mixture was incubated at 37 °C for two hours and the digested samples were separated by electrophoresis through a 2.5% agarose gel and visualized by staining with ethidium bromide.

Statistical analysis

Values are given as the mean and standard deviation. Univariate statistical analysis was performed using Student's unpaired test and the χ^2 test. Multivariate unconditional logistic regression was used to estimate odds ratios (OR) and to adjust for potential confounders. The statistical power was estimated with appropriate software (SPSS). Although the number of males was only 40, it should be emphasized that the statistical power (81.5%) was able to demonstrate an effect.

The likelihood ratio test was used to assess statistical significance. Product-terms in the logistic models were used to assess interactions (effect modification) between the ADRB2 polymorphism and gender, and between this polymorphism and HDL-levels. HDL-levels were dichotomized in two categories using 1.5 mmol/L as the cut-off point.

Results

As expected, cases differed from controls in regard to several obesity risk factors such as body fat, WHR, high blood pressure, insulin and leptin levels (Table 1). The prevalence of the 27Glu polymorphism was slightly higher among cases (51% heterozygotes and 17% homozygotes) than among control subjects (43% heterozygotes and 16% homozygotes). Among males, cases with abdominal obesity (n = 16) had a significantly

Table 1 Baseline characteristics of the study groups.

	Cases (n = 112)		Controls (n = 127)	
	Mean	(SD)	Mean	(SD)
Male (n)	20		20	
Age (years)	44.1	(9.6)	37.4	(8.4)
Weight (kg)	96.4	(15.2)	59.5*	(8.9)
Body mass index (kg/m^2)	37.7	(5.3)	22.0*	(1.8)
Blood pressure (mmHg)				
Systolic	139.7	(16.8)	108.4*	(11.7)
Diastolic	88.1	(10.3)	67.6*	(8.8)
Waist-hip ratio	0.93	(0.05)	0.77*	(0.04)
Body fat %	42.7	(7.0)	27.8*	(4.8)
Glucose (mM/L)	5.9	(1.7)	5.1*	(0.5)
Total cholesterol (mM/L)	5.5	(1.0)	5.0*	(0.9)
HDL-cholesterol (mM/L)	1.3	(0.3)	1.7*	(0.3)
LDL-cholesterol (mM/L)	3.5	(0.9)	3.0*	(0.8)
Total /HDL cholesterol ratio	4.3	(1.1)	3.0*	(0.8)
Triglycerols (mM/L)	1.4	(0.7)	0.7*	(0.3)
Leptin (ng/ml)	34.4	(31.2)	7.9*	(7.4)
Insulin (pM/L)	167.6	(165.2)	53.7*	(32.1)

Values are shown as mean and standard deviation (SD).
*$p < 0.05$ as compared to cases (obese)

higher prevalence of the 27Glu polymorphism (80 % vs. 50 %, p = 0.026) than controls (n = 10), while no differences in the proportion of carriers of the 27Glu between cases and controls were detected among female participants (n = 199). Among male subjects with abdominal obesity, 30 % of 27Glu carriers were homozygous for the mutation (n = 6), but only 15 % were homozygous among male controls (n = 3). When we assessed the effects of the 27Glu allele of the ADRB2 gene on the risk of developing abdominal obesity (Table 2) using a logistic regression model, we detected a strong association between the 27Glu polymorphism and obesity in male subjects (OR = 4.08, 95 % CI: 0.98–16.3), but no such association was apparent among women. After adjustment for age, a statistically significant odds ratio of even a higher magnitude (OR = 10.31, 95 % CI 1.37–76.8; p = 0.023) was found for males. When we introduced a product term (gender x 27Glu allele) to assess effect modification in the multivariate logistic regression model, a borderline statistically significant interaction (p = 0.056) between gender and the ADRB2 mutation was found (Table 2).

However, when we further examined the association between abdominal obesity and the ADRB2 variant according to HDL-cholesterol levels (Table 2), another effect modification by the HDL-cholesterol levels was apparent. We found an OR = 2.87 (95 % CI: 1.09–7.50) after adjustment for age and gender among participants having the HDL-cholesterol levels in the lower category (< 1.5 mmol/L), whereas no such association was found among those with higher HDL-levels. When a product-term (HDL-cholesterol levels x 27Glu allele) was introduced in the model, a statistically significant interaction (p = 0.003) between HDL-cholesterol levels and the ADRB2 mutation was also apparent.

Discussion

A case-control study to assess the association of abdominal obesity (both high BMI and high WHR) with the 27Glu mutation of the ADRB2 gene was conducted. Our study shows for the first time that the 27Glu allele of the ADRB2 gene is a gender-dependent genetic risk factor for abdominal obesity. Furthermore, the fact that the subgroup of males and those having lower HDL-cholesterol levels show statistical differences supplies relevant information to be applied in specific situations as has been found for other polymorphisms [3].

In the present work, we have included men and

Table 2 Risk of abdominal obesity associated with β_2-adrenergic receptor gene mutation: effect modification by gender and HDL-cholesterol levels.

		Risk of abdominal obesity (BMI > 30 and WHR > 0.85) for 27Glu allele[a]			
	n	Crude OR (95 % CI)		Age-adjusted OR (95 % CI)	
Sex					
Male	40	4.08	(0.98–16.3)	10.31	(1.37–76.8)
Female	199	1.21	(0.68–2.16)	1.10	(0.60–2.03)
	n	Crude OR (95 % CI)		Age and gender adjusted OR (95 % CI)	
HDL-Cholesterol (mmol/L)					
< 1.5	114	2.15	(0.91–5.05)	2.87	(1.09–7.50)
≥ 1.5	125	0.81	(0.35–1.87)	0.61	(0.24–1.52)
Overall	239	1.46	(0.86–2.49)	1.40	(0.80–2.47)

[a] Cases had a BMI > 30 and WHR > 0.85 and controls had BMI < 25 and WHR < 0.85.
Among males, cases with abdominal obesity (n = 16) had a significantly higher prevalence of the 27Glu polymorphism (p = 0.026) than controls (n = 10).

women and cases were defined as having both BMI > 30 and WHR > 0.85, whereas controls had a BMI < 25 and WHR < 0.85. To our knowledge, this is the first case-control study devised to specifically analyze the risk of abdominal obesity. This design had both strengths and weaknesses. In our study, on the positive side, sources of cases and controls were two well-characterised groups within the same source population, response rates were relatively acceptable (65 % for cases and 75 % for controls), and the interviews were all conducted in a medical environment with little or no time pressure. On the negative side, there were slight differences between cases and controls regarding the distribution of age and gender because we did not follow a matched case-control design. However, since age and gender were always taken into account and controlled for using multivariate logistic regression models for all estimates of association, we were in a position to correctly address their independent effects on the risk of abdominal obesity and to assess effect modification of the 27Glu mutation by these and other factors, using product-terms [16]. Also, it should be emphasized that this investigation involved a cross-sectional study giving information about association, but not on causality.

The prevalence of the 27Glu variant was slightly higher in cases (68 %) than in controls (59 %) and comparable data have been obtained in other Caucasian populations [6, 12]. Interestingly, after stratification by gender, we observed an uneven distribution within males, with the number of male carriers of the 27Glu allele substantially higher among cases (80 %) than among controls (50 %). After adjusting for age, male carriers of the 27Glu allele had a significantly higher (10x) *odds ratio* of abdominal obesity than non-carriers.

Other investigators [4, 5] previously suggested a gender-dependent effect of the 27Glu allele in regard to obesity, although results were controversial. Our finding of an interaction between the ADRB2 polymorphism and gender concerning abdominal obesity may clarify the different role of this polymorphism within males and females [4, 13, 14]. Also this contribution may help to explain some of the discrepancies reported in the literature when the breakdown by gender was not taken into account. In fact, the occurrence of abdominal obesity is different in men and women [15] as well as the role of the ADRB2 appears as dependent on gender [4].

We also found that participants with HDL-cholesterol levels lower than 1.5 mmol/l had a significantly higher risk of abdominal obesity linked to the 27Glu allele of the ADRB2 gene regardless of age and gender (OR = 2.87, 95 % CI 1.09–7.50). Other studies [17–19] have also found an association between HDL-cholesterol levels and different gene polymorphisms (PPARγ2, lipoprotein lipase, etc.), but this is apparently the first study to report a significant interaction ($p = 0.003$) between HDL-cholesterol levels and this mutation in the ADRB2 gene.

The relevance of this study lies in the fact that abdominal obesity may be important to explain the different risk between females and males, whichs give further value to previously reported studies [4, 20] and about potential therapeutic strategies [20, 21].

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