RAPID COMMUNICATION

The absence of polymorphisms in ADRB3, UCP1, PPARγ, and ADIPOQ genes protects morbid obese patients toward insulin resistance

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ABSTRACT. ***Background and aims:*** **The insulin resistance (IR) is a major metabolic impairment in severe obesity, a multifactorial disease in which the importance of the effect of single nucleotide polymorphisms (SNP) associations in different rather than individual genes was established. The aim of this study was to test the predictive value of presence/absence of polymorphisms/variants in β3-adrenergic receptor (ADRB3), uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor γ (PPARγ), and adiponectin (ADIPOQ) genes in diagnosing the IR in obesity.** ***Subjects and methods:*** **We studied 112 (40 males, 72 females) severely obese (body mass index: 48.5±7.5 kg/m²) subjects recruited from the outpatient obesity clinic of Federico II University Hospital in Naples. Genomic DNA was extracted from peripheral leukocytes with a commercial kit. The gene polymorphisms Trp64Arg in ADRB3, -3826 A>G in UCP1, Pro12Ala in PPARγ, and c.268G>A, c.331T>C, and c.334C>T in ADIPOQ were characterized by TaqMan assay or by direct sequencing (ADIPOQ).** ***Results and conclusion:*** **Our results demonstrate that -3826A>G UCP1 polymorphism is associated with IR in morbid obesity. Further, the lack of any polymorphisms, Trp64Arg in ADRB3 and/or -3826 A>G in UCP1 and/or Pro12Ala in PPARγ and/or c.268G>A, c.331T>C and c.334C>T in ADIPOQ, appears a useful prognostic factor (NPV=100%) toward the IR onset in these obese patients representing a further parameter for an earlier and appropriate therapy.**

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INTRODUCTION

During the last decades, an alarming increase in the prevalence of obesity ("globesity") has been observed worldwide among both children and adults (1). Obesity is associated with increased risk for several morbidities including diabetes mellitus, dyslipidemia, cardiovascular diseases, and some cancers (2). Weight gain due to increased fat mass is the consequence of a long-standing imbalance between energy intake and energy expenditure, influenced by multiple and complex interactions between genes and environment (3). High-calorie diet and sedentary lifestyle are considered to be the main environmental factors leading to weight gain (1). On the other hand, genetic factors affecting appetite, energy expenditure, and adipocyte metabolism may predispose individuals to develop obesity (4). Epigenetic interactions have also been described as predisposing factors for abdominal obesity and related diseases (5). Among the most common obesity-related complications, the metab - olic syndrome (MS) has a high prevalence in severe obesity representing a cluster of metabolic alterations, such as altered levels of adipokines, hyperglycemia, dyslipidemia and/or hypertension (6, 7).

Some genes directly interact with glucose and lipid metabolism and can therefore determine a greater prevalence of metabolic alterations (8). Polymorphisms/variants in genes encoding molecules known to be involved in energy expenditure, fat metabolism, and insulin sensitivity such as β3-adrenergic receptor (ADRB3) and uncoupling protein 1 (UCP1) or other genes related to adipocytes differentiation and anti-inflammatory mechanisms, like peroxisome proliferator-activated receptor γ (PPARγ), and adiponectin (ADIPOQ), have been extensively studied (4, 8).

Single nucleotide polymorphisms (SNP) in the abovementioned genes have been reported in healthy and unhealthy subjects (4, 9, 10). Particularly, our group showed the Trp64Arg ADRB3 polymorphism to be related to insulin resistance (IR) in severe obesity (11), whereas UCP1 -3826 A>G polymorphism and several SNP in ADIPOQ predisposed to MS (12, 13).

Since the importance of the SNP associations in different genes related to complex diseases, rather than individual genes, in this study, our aim was to evaluate the global influence of polymorphisms Trp64Arg in ADRB3 gene, Pro12Ala in PPARγ gene, -3826 A>G in UCP1 gene, and 3 SNP previously described in association with IR (c.268G>A,

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c.331T>C and c.334C>T) in ADIPOQ gene, on the IR in a morbid obese population from Southern Italy.

SUBJECTS AND METHODS

Subjects

The study population consisted of 112 (40 males, 72 females) severely obese non-diabetic subjects recruited from the outpatient obesity clinic of the Department of Medicine, Federico II University Hospital in Naples. All subjects had normal liver, kidney and thyroid function, and none had a history of excessive alcohol intake. None was taking anti-hypertensive drugs or substances known to affect resting metabolic rate, or glucose or lipid metabolism.

Anthropometric and metabolic measurements

Weight and height were measured in standardized conditions and the body mass index (BMI) was calculated. Systolic and diastolic blood pressures were measured after the subject had rested for 5 min in a sitting position. After a 10-h overnight fast, blood was collected and centrifuged. Plasma glucose was measured by the hexokinase method adapted for an autoanalyzer. Total cholesterol, triglycerides, and HDL cholesterol were determined by standard enzymatic methods. MS was diagnosed considering the combination of 3 out of 5 risk factors according to American Heart Association (AHA) criteria (6). Serum insulin was measured by the chemiluminescence method (Immulight 2000; Italy). IR was estimated according to the homeostasis model assessment (HOMA-IR) method from fasting glucose and insulin concentrations, according to the formula: insulin (mU/ml) × glucose (mmol/l)/22.5. We used to classify as IR the threshold of 4.65, previously indicated for obese subjects (14).

Genotyping

Genomic DNA was extracted from peripheral leukocytes with a widely used procedure (Nucleon BAAC-2, Amersham, UK).

We identified Trp64Arg for ADRB3, -3826 A>G for UCP1 and various SNP (mutations) for ADIPOQ, respectively as described elsewhere (11-13).

The PPARγ Pro12Ala polymorphism was identified by the real-time TaqMan method (Applied Biosystems, USA) and two fluorescent probes. We used the Primer Express program to design the PCR primers forward 5'-TGACTCATGGGTGTATTCACAA-3', reverse 5'-CAAACACAACCTGGAAGACAAA-3'; MGB TaqMan probes VIC-TCCTATTGACCCAGAAAGCGA-Q-MGB and FAM-TCCTATTGACGCAGAAAGCGA-Q-MGB where MGB is the minor groove binder, a molecule that stabilizes the duplex DNA probe thereby increasing the ability of the hybridization probe to discriminate the single-nucleotide polymorphism. The protocol was the same as previously described (11).

Statistics

Allele frequencies were calculated by allele counting and the departure from Hardy-Weinberg expectation was evaluated by the χ^2 test using the Haploview 3.2 software with default p value cut-off (15). The χ^2 test was also used to test any difference in allele SNP frequency between obese patients and controls. Multiple comparisons were corrected by using the Bonferroni test. Binomial logistic regression analysis was used to investigate the association between the genetic and clinical characteristics and the presence of IR. To explore the possibility of missing a potential association due to the loss of information introduced by the binary categorization of the HOMA in the logistic analysis (HOMA > or <4.65), a multiple linear regression analysis was performed using the continuous HOMA as dependent variable for the same set of independent variables used in the logistic. Both forward and backward procedures were used for model selection and gave concordant results. Differences were considered statistically significant with a *p*-value <0.05. Statistical analyses were carried out with the PASW package for Windows (Ver.18; SPSS Inc. Headquarters, Chicago, Ill).

RESULTS AND CONCLUSION

The mean age of obese patients in the study was 32.7±10.5 yr and mean BMI was 48.5±7.5 kg/m^2. According to the used threshold of HOMA=4.65, 50/112 (45%) of obese subjects were IR (+). Table 1 reports the frequen-

Table 1 - *Genotype frequencies of the studied polymorphisms according to the presence/absence of insulin resistance in 112 severe obese patients.*

Gene	Genotype	Total obese population no. (%)	Insulin resistance		χ^2 test *p*
			IR (-) no. (%)	IR (+) no. (%)	
ADRB3	WT	102 (91.1)	57 (50.9)	45 (40.1)	ns[b]
	Pol[a] – Trp64Arg	10 (8.9)	5 (4.5)	5 (4.5)	
UCP1	WT	29 (25.9)	23 (20.5)	6 (5.4)	0.003
	Pol[a] – -3826 A>G	83 (74.1)	39 (34.8)	44 (39.3)	
PPARγ	WT	86 (76.8)	47 (42.0)	39 (34.8)	ns[b]
	Pol[a] – Pro12Ala	26 (23.2)	15 (13.4)	11 (9.8)	
ADIPOQ	WT	105 (93.8)	58 (51.8)	47 (42.0)	ns[b]
	Pol[a] – c.268G>A, c.331T>C, and c.334C>T	7 (6.3)	4 (3.6)	3 (2.6)	
4 genes[c]	WT	11 (9.8)	11 (9.8)	0 (0)	0.001
	Pol[a]	101 (90.2)	51 (45.5)	50 (44.7)	

[a]Polymorphic (heterozygous + homozygous) genotype; [b]not statistically significant difference of genotypes distribution between insulin resistance yes/no; [c]subjects were considered polymorphic if bearing at least 1 studied single nucleotide polymorphism in the investigated genes.

Table 2 - *Results of multiple linear regression analysis with homeostasis model assessment index as dependent variable.*

Models	*p*	R^2	Partial R^2	B coefficient	95% CI
1[a]	<0.0001	0.121	0.121	2.3	1.2-3.5
2[b]	0.007	0.169	0.048	1.8	0.5-3.1
3[c]	0.015	0.201	0.032	2.0	0.8-3.9

Predictors: [a]male sex; [b]male sex + UCP1 polymorphism; [c]male sex + UCP1 polymorphism + ADRB3 polymorphism. CI: confidence interval.

cies of the genotypes observed, at level of the investigated genes, in the total obese population and in the two groups IR (+) and IR (-) obese patients. The -3826A>G UCP1 polymorphic genotypes were more present among the IR (+) (88%) than in IR (-) (63%) obese patients [*p*=0.003; odds ratio (OR): 4.3, 95% confidence Interval (CI): 1.6-11.7]. All IR (+) obese patients were polymorphic in one or more genes (*p*=0.001) (Table 1); in addition, the absence of any assessed polymorphism had an high negative predictive value (NPV=100%) for IR.

The binomial logistic regression analysis [dependent variable: IR (+)/IR (-); independent variables: presence/absence of polymorphisms in the studied genes, biochemical variables (triglycerides, total and HDL cholesterol, aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transferase)], showed, after correction for confounding variables (BMI, age, sex), a significant positive association between the IR (+) with male sex (*p*<0.0001; OR/95% CI: 10.3/3.0-35.2), UCP1 polymorphic genotype (*p*=0.001; OR/95% CI: 17.8/3.4-92.7) and triglycerides (*p*=0.004; OR/95% CI 1.01/1.00-1.02). By multiple linear regression analysis, the final model resulted in the addition of ADRB3 polymorphism to male sex and UCP1 as further significant factor, being the overall adjusted R square equal to 0.201 (*p*=0.015) (Table 2).

The studies on obesity and its association with metabolic alterations are complicated by the highly heterogeneous genetic basis; in fact, several genes are involved and contribute to the pathogenesis of this complex disease, but while each single gene has only a small effect, a combined effect of all genes results in pathologic phenotypes.

In conclusion, our results demonstrate that -3826A>G UCP1 polymorphism, that we previously demonstrated to be associated with MS (12), is also associated with IR (+) in morbid obesity. Further, the lack of polymorphisms at level of the Trp64Arg in ADRB3 and/or the -3826 A>G in UCP1 and/or the Pro12Ala in PPARγ and/or the c.268G>A, c.331T>C and c.334C>T in ADIPOQ, appears an useful prognostic factor (NPV=100%) toward the IR onset in these obese patients. Hence, our findings could suggest additional criteria to characterize the clinical background in morbid obesity in order to design targeted therapies based on specific metabolic alterations of the single subjects. This preliminary observation requires further investigation in larger population studies.

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