The Val\textsuperscript{81} missense mutation of the melanocortin 3 receptor gene, but not the 1908C/T nucleotide polymorphism in lamin A/C gene, is associated with hyperleptinemia and hyperinsulinemia in obese Greek Caucasians

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ABSTRACT. Obesity-related phenotypes have been linked to human chromosomes 1q21 and 20q13, regions where the lamin A/C gene (LMNA) and the melanocortin 3 receptor gene (MC3R) map, respectively. Recently, a common single nucleotide polymorphism (SNP) in LMNA (1908C/T) was associated with plasma leptin and obesity indices in aboriginal Canadians, but these associations have not yet been explored in other populations. In contrast, no significant associations of MC3R variants with obesity have been detected, although a significant association with hyperinsulinemia has been reported in Caucasian populations. We investigated the associations between the LMNA 1908C/T variant and the 241G/A variant of the MC3R gene (Val\textsuperscript{81}Ile missense mutation) and body composition, as well as plasma leptin and insulin levels, in two samples of unrelated healthy Greek subjects. A group of 112 young non-obese subjects, and a group of 116 adult women with a body mass index (BMI) ranging from 23.2 to 47.7 kg/m\textsuperscript{2} were studied cross-sectionally. We found no significant association of the LMNA 1908C/T and a borderline significant association of MC3R 241G/A SNPs with body composition variables, in the entire study sample. However, unlike the LMNA 1908C/T genetic variation, the MC3R 241G/A genetic variation was significantly associated with hyperleptinemia and hyperinsulinemia in obese subjects, and there was evidence of interaction between this polymorphism and fat mass or BMI in predicting hyperinsulinemia. Our results suggest that the LMNA 1908C→T substitution and the Val\textsuperscript{81}Ile mutation of the MC3R gene are unlikely to be major predictors of body composition in Greek Caucasians, but the latter genetic variation may predispose obese subjects to develop insulin and leptin resistance. Future studies are needed to confirm these data and assess whether individuals carrying this mutation are more resistant to weight-reducing and insulin-sensitizing treatments.


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INTRODUCTION

Obesity has a strong genetic component with a few major and more than 200 minor genes having been implicated in its pathogenesis (1). Among several candidate genes for obesity, two, the gene encoding the nucleophilic A-type lamins (LMNA) and the melanocortin 3 receptor gene (MC3R), were recently considered as plausible candidates based on evidence from animal experiments and/or linkage to obesity, insulin resistance and hyperinsulinemia in humans (2-5). Hegele et al. (6) identified a common single nucleotide polymorphism (SNP) in exon 10 of LMNA (1908C/T), which was associated with increased anthropometric measurements and plasma leptin concentrations in a population of aboriginal Canadians. This association of the LMNA 1908T allele was further confirmed in an independent, genetically distinct aboriginal population, the Canadian Inuit (7).
Recently, the LMNA 1908C→T substitution was associated with reduced age, sex and percent fat mass (%FM) adjusted mean sc abdominal adipocyte size in Pima Indians (8), but Wolford et al. (9) found no evidence of association of this polymorphism with body mass index (BMI), leptin concentrations or diabetes in this population. Whether the LMNA 1908T allele may be associated with obesity in Caucasians has not yet been studied. The MC3R gene has also been proposed as a candidate for susceptibility to obesity, due to its chromosomal localization within a region linked to obesity-associated traits and plasma insulin levels in human populations (4, 5), as well as two recent reports describing increased fat mass (FM) and reduced lean mass in MC3R gene deficient mice (10, 11). Two common missense mutations have been reported to date in the MC3R coding region in humans (12-14) and one extremely rare that has been found in heterozygosity in two obese members of a Singaporean family (15). These two common missense mutations, namely a C→A and G→A variations resulting in a lysine to threonine (Lys→Thr) and a valine to isoleucine (Val→Ile) amino acid changes at codons 6 and 81 of the MC3R respectively, were found in complete linkage disequilibrium and were not associated with diabetes or BMI in Type 2 diabetic French (13) and obese Finnish (14) subjects. In addition, the Val→Ile mutation (241G/A variant) was not significantly different between 124 extremely obese (BMI>40 kg/m²) and 85 average weight (BMI<27 kg/m²) Caucasian and Afro-American women (12). Although based on these observations the Val→Ile mutation is unlikely to be a key factor in human body weight control in extremely obese subjects; it has been hypothesized that this polymorphism may influence adiposity or obesity indices in populations with less extreme obesity or different ethnic background (12), and/or that it may be associated with hyperleptinemia and hyperinsulinemia (13, 14).

We thus explored potential associations between body composition variables, as well as plasma leptin and insulin levels, and the LMNA 1908C/T and MC3R 241G/A polymorphisms in two independent groups of healthy Greek subjects; a group of 112 non-obese young subjects, and a group of 116 adult women with a BMI ranging from 23.2 to 47.7 kg/m².

MATERIALS AND METHODS
Subjects
A total of 228 unrelated healthy subjects participated in the two studies presented herein: 112 young subjects with a normal range of adiposity (group A) and 116 adult women (group B). Subjects in group A were healthy students for whom DNA and relevant clinical information were available, and who were consecutively enrolled in a study designed to evaluate the nutritional status of young adults in Greece. Subjects in group B were recruited through advertisement in local newspapers and magazines, as part of a study designed to assess body composition parameters in adult Greek women. A total of 131 women responded but only those (no. =116) who were healthy, without known history of diabetes or participation in a weight loss program in the last six months were enrolled in the present study. Before undergoing a health examination, all subjects completed specific questionnaires on demographic characteristics and general health status. None of the study subjects was taking any medications or had a history of a known disease. Fasting blood samples were collected from all subjects. Plasma was immediately frozen at −70°C for analyte determination and the buffy coats of nucleated cells were used for the extraction of DNA. The study protocol was approved by the Ethics Committee of Harokopio University and all subjects gave informed consent prior to participating in the study.

Anthropometric assessment and hormone measurements
Standardized procedures were used in performing anthropometric measurements, as previously described (16). For all subjects, weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively. The BMI was calculated as weight (kg) divided by height (m) squared. Waist, hip and arm circumferences were also measured to a precision of 0.1 cm, and the waist-to-hip ratio (WHR) was calculated. In addition, triceps, biceps, subscapular and suprapiole skinfolds were measured twice by the same observer on the right side of the body to a precision of 0.2 mm, and the average of the two measurements was used. Body composition was assessed in both groups by bioelectrical impedance analysis as previously described (16), using the age and gender specific regression equations developed by Deurenberg et al. (17) (for group A) and the fatness specific ones developed by Segal et al. (18) (for group B). Plasma leptin (ng/ml) and insulin (µU/ml) concentrations were measured using radioimmunoassay (leptin: Linco Research, St Louis, MO; insulin: DSL, Wester, Texas), and the limits of detection were 0.5 ng/ml and 1.3 µU/ml, respectively.

DNA analysis
Genomic DNA was extracted from leucocyte nuclei as previously described (15). The LMNA 1908C/T genotype was determined after restriction fragment length polymorphism analysis of PCR amplified DNA, as described by Hegele et al. (6). The MC3R 241G/A genotype was determined after amplification with primers 5′ to 3′: TGGAGGGAATTGTCTTTTCGT; TTTCCAGCAGACTGAGTGC (described by Li et al. (12), over 34 amplification cycles and an annealing temperature of 60°C. The 256-bp amplification product was then digested with Mnl I (13) and electrophoresed in 5% agarose gels. Digestion of the 241G allele gave four fragments with sizes 122, 50, 47 and 37 bp, whereas digestion of the 241A allele gave three fragments with sizes 122, 97 and 37 bp.

Statistical analysis
Statistical analyses were performed using the SPSS statistical software (SPSS for windows, release 10.0.5). Allele frequencies were estimated by the gene counting method, and differences in genotype distributions or allele frequencies were tested by chi-square analysis. Associations of quantitative traits with geno-