Research paper

The combined effect of MC4R and FTO risk alleles on childhood obesity in Greece

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ABSTRACT

OBJECTIVE: Single nucleotide polymorphism (SNP) risk alleles of obesogenic genes, such as the fat mass and obesity-associated protein (FTO) and the melanocortin-4 receptor (MC4R) gene, have been described in both paediatrics and adult populations. The aim of this study was to investigate the combined effect of FTO and MC4R risk alleles on the obese phenotype and metabolic profile of young children and adolescents of Greek origin. DESIGN: One hundred and fifty-three overweight and obese Greek children (mean age 11.08±2.23 years) and 151 age-matched normal-weight controls were enrolled. Along with anthropometric and standard biochemical profile genetic analysis for the high-risk rs9939609 A allele of the FTO gene and the high-risk C allele of the rs17782313 polymorphism downstream of the MC4R gene were investigated in all participants. RESULTS: The combined presence of three or more high-risk alleles of both the FTO and MC4R genes confers a 4-fold higher risk for obesity in children and adolescents of Greek origin, although these risk alleles have no impact on the metabolic alterations observed in these obese children and adolescents. CONCLUSIONS: There is a synergistic effect of the high-risk alleles of the FTO and MC4R genes on the obese phenotype, while no impact on the metabolic abnormalities was observed in Greek obese children and adolescents.

Key words: BMI, Childhood obesity, FTO, MC4R, Metabolic abnormalities, SNP

INTRODUCTION

Childhood obesity has evolved into a worldwide

epidemic. It has been estimated that in 2010, 43 million children aged <5 years were overweight or obese and that by 2020 this percentage will have risen to 60 million children.[1] Contrary to prior common beliefs, it has been proved that "heavy" children will grow into unhealthy, obese adults with a plethora of medical conditions, this rendering the current epidemic a global health crisis on multiple levels. Greece has the highest percentage of overweight and obese adolescents in Europe, at 44% and 11%, respectively.[2]

The radical changes of lifestyle during the past 50 years have undoubtedly created an "obesogenic" environment characterized by sedentary habits and energy-dense palatable foods that are accountable for the present explosion in obesity. However, not everyone becomes overweight. Studies on heritability of human adiposity have estimated that genetic factors contribute up to 40-70% of the variance of adiposity.[3,4] Genome Wide Association Studies (GWAS) have contributed to researching genetic variations of multiple loci. Still, in the case of common obesity single nucleotide polymorphisms (SNPs) variations can explain only up to 1.5% of the variance in BMI.[4]

The first "obesogenic" SNP to be replicated and validated was the fat mass and obesity-associated (FTO) gene consisting of nine exons on chromosome 16 (16q12.2).[5] It encodes a 2-oxoglutarate-dependent nucleic acid demethylase[6] and is found in higher concentrations in the hypothalamus. Its concentration in the arcuate nucleus fluctuates depending on the fasting state. Even though its physiological function has not yet been elucidated, evidence so far associates FTO variants with hyperphagia of calorie-dense foods.[6,7] Recent studies reveal a strong correlation between the rs9939609 A allele of the FTO and childhood obesity.[8,9] In this SNP, located in the first intron, threonine (T) is substituted by alanine (A) (T->A), this leading to a possible protein dysfunction that ultimately results in increased body weight.

Additionally, in humans gain-of-function mutations of the MC4R gene have been described as a relatively frequent genetic finding in cases of obesity.[10-12] At least 72 polymorphisms of the MC4R gene have been described so far with a small number of them leading to formation of a defective, mutated MC4R. Among these mutations some seem to cause intracellular retention of the deformed protein, while others cause abnormal ligand binding or ligand/receptor defects.[12-15] The common SNP rs17782313 substituting a threonine amino acid (T) for cysteine (C) (T->C), lying 188 kb downstream of the MC4R gene, has recently been associated with increased body mass index (BMI) during childhood, affecting energy intake as well as energy expenditure, while in adults it has been associated with insulin resistance and diabetes risk.[16-22]

Aim of the study

The aim of the study was to investigate the impact of the high-risk C allele of the rs17782313 polymorphism of the MC4R gene and of the high-risk A allele of the rs9939609 polymorphism of the FTO gene and their combined effect on the BMI and metabolic traits of overweight and obese children and adolescents in Greece.

MATERIALS AND METHODS

Subjects and Methods

Overweight and obese subjects

One hundred and fifty-three (153) obese Greek children examined in the Obesity Outpatient Clinic of the Division of Endocrinology, Diabetes and Metabolism of the First Dept. of Paediatrics of the University of Athens in "Aghia Sofia" Children's Hospital from 2006 to 2009 who were willing to participate were included in the study. Subjects were classified as overweight, obese and normal-weight according to the International Obesity Task Force (IOTF) cut-off limits.[23] In order to avoid a probable heterogeneity in the allele frequencies between Greek and non-Greek Caucasians, only children of Greek origin were included in the present study. Exclusion criteria were underlying chronic illness, chronic medication and presence of a genetic syndrome, chromosomal disorders, mental disorders or pre-existing psychopathology.

Normal-weight subjects (controls)

Normal-weight children included in this study as controls were recruited for the GENDAI study.[24] Briefly, normal-weight children were recruited from fifth and sixth grade classes of several elementary schools in the region of Attica, Greece. The selected schools (40 schools in a total of 593 in the region) were all in socioeconomically similar urban ureas. The controls were age-matched with the overweight/obese group. The study was approved by the Ethical Committee of "Aghia Sofia" Children's Hospital and subjects were enrolled in the study only after informed written consent had been obtained from their parents or guardians.

Anthropometric data

Anthropometric measurements were obtained

using classical methods. Briefly, all children were dressed in light clothes. For height measurement they were placed in the Frankfurt plane. Height was measured in centimeters and weight in kilograms. Waist circumference was measured using a soft tape around the 12th rib, hip circumference around the iliac crest and the waist-to-hip ratio was calculated dividing the two measurements. BMI was calculated as the ratio of weight/height^2 (kg/m^2). Classification of children as normal-weight, overweight and obese met the criteria defined by the IOTF.[23]

Blood chemistry

Blood was collected after an overnight fast at 8:00 a.m. The fasting glucose (Glu), total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations in serum were assayed using the ADVIA 1800 Clinical Chemistry Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), while insulin levels were determined with an electrochemiluminescence immunoassay on the Roche ELECSYS 2010 immunoassay analyzer (Basel, CH).

The HOMA insulin resistance (HOMA-IR) index was calculated according to the formula: HOMA-IR Index = [(Fasting Insulin) × (Fasting Glucose)]/161, with insulin concentration expressed in pmoles/l and glucose in mmoles/l.

Genotyping SNPs rs17782313 of MC4R and rs9939609 of FTO

For the genetic analysis, DNA was extracted from white blood cells from whole blood sampling using standard methods. Single nucleotide polymorphisms rs17782313 and rs9939609 of the MC4R and FTO genes, respectively, were analyzed after Real Time PCR amplification using the LightCycler 2.0 (Roche, Mannheim, Germany). The Real Time PCR LightCycler assay is based on hybridization probes labeled with fluorescent dyes that allow fluorescence resonance energy transfer.[25] SNP genotyping was carried out using melting curve analysis. Sequences of primers and probes used for Real Time PCR with the LightCycler 2.0 are available upon request.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Association statistics were calculated using PLINK software.[26] Continuous variables are presented as mean values ± standard deviation. The statistical test used to delineate statistical significance (p-value) in normally distributed continuous variables between groups was the analysis of variance (ANOVA) test, as depicted in Table 1. For non-normally distributed variables, the natural logarithmical transformed values were used. Linear regression was performed to test the hypothesis of association between polymorphisms as predictors and their effect on anthropometric indices after adjustment for potential confounders (age, gender) (Table 2). Logistic regression was performed to determine the independent effect of risk alleles of MC4R and FTO on the relative likelihood of obesity.

Table 1. Descriptive characteristics of normal weight and overweight/obese subjects

	Normal weight	Overweight/Obese	p-value
Number	151	153	
Age	10.96 ± 0.57	11.08 ± 2.23	0.494
Weight (kg)	37.96 ± 5.7	66.43± 19.6	<0.001
Weight Z-score	-0.48 ± 0.78	4.2 ± 2.77	<0.001
Height (m)	1.46 ± 0.70	1.49 ± 0.12	0.003
Height Z-score	-0.12 ± 0.98	0.56 ± 1.46	<0.001
BMI (kg/m^2)	17.75 ± 1.5	29.27 ± 5.6	<0.001
BMI Z-score	-0.44 ± 0.44	2.95 ± 1.65	<0.001
Waist circumference (cm)	61.99 ± 5.23	88.73 ± 14.64	<0.001
Waist-Hip ratio	0.77 ± 0.04	0.89 ± 0.09	<0.001
Waist-Height ratio	0.42 ± 0.032	0.59 ± 0.082	0.000
Birth weight (g)	3202 ± 459	3366 ± 479	0.006
Systolic blood pressure (mm Hg)	118 ± 12.16	116 ± 16.8	0.420
Diastolic blood pressure (mm Hg)	74.02 ± 10.8	75.83 ± 10.82	0.234
Insulin (uU/ml)	6.54 ± 3.51	14.46 ± 6.77	<0.001
Glucose (mmol/l)	4.70 ± 0.38	4.70 ± 0.41	0.935
HOMA-IR	1.38 ± 0.79	3.06 ± 1.49	<0.001
Cholesterol (mg/dl)	186 ± 27	170 ± 33	<0.001
HDL-C (mg/dl)	54 ± 9.7	46 ± 10	<0.001
LDL-C (mg/dl)	120 ± 22	105 ± 27	<0.001
Triglycerides (mg/dl)	59 ± 18	79 ± 31	<0.001

Table 2. Linear regression analysis of the impact of the MC4R rs17782313 C allele and the FTO rs9939609 A allele on anthropometric indices and metabolic traits in all subjects

	MC4R rs17782313 C allele			FTO rs9939609 A allele		
	Mean	SE	P-value	Mean	SE	P-value
Weight	3.998	1.809	0.0279	3.603	1.418	0.011
Weight Z-score	0.7053	0.3041	0.0210	0.6168	0.2387	0.01
Height	-0.0009	0.0073	0.8984	0.0117	0.0057	0.04
Height Z-score	0.0503	0.1235	0.6842	0.1828	0.0965	0.059
BMI	1.737	0.688	0.0122	1.346	0.5426	0.013
BMI Z-score	0.5066	0.2063	0.0147	0.3813	0.1629	0.02
Waist circumference	2.821	1.719	0.102	3.58	1.391	0.01
Waist-Hip ratio	0.0094	0.0091	0.3033	0.0179	0.0074	0.016
Waist-Height ratio	0.0189	0.0107	0.0779	0.0201	0.0087	0.021
Birth weight	-0.357	50.63	0.9944	5.26	41	0.895
Head circumference	2.243	1.334	0.094	2.227	1.086	0.041
SBP	-1.921	1.742	0.2714	2.186	1.405	0.121
DBP	0.355	1.12	0.9766	0.0232	0.989	0.9813
INS	0.859	0.673	0.2029	0.4711	0.5439	0.39
INS (LN)	0.0702	0.068	0.3033	0.05071	0.0555	0.36
GLC (MMOL)	0.031	0.0405	0.4469	0.0003	0.0326	0.9927
HOMA IR	0.201	0.1472	0.1743	0.1137	0.1196	0.342
HOMA (LN)	0.0772	0.0706	0.2747	0.05417	0.0578	0.349
CHOL	4.305	3.211	0.1812	-0.1612	2.557	0.949
HDL-C	1.168	1.084	0.282	-0.811	0.872	0.353
LDL-C	2.091	2.714	0.4416	-1.071	2.169	0.622
TGS	1.347	2.764	0.6264	0.9453	2.217	0.670
TGS (LN)	0.0034	0.0375	0.928	0.0185	0.03	0.539

*BMI, BMI z-score and weight were adjusted for age and gender; birth weight and birth weight z-score were adjusted for gender; waist circumference and waist-hip ratio were adjusted for age, gender and BMI; glucose, insulin, HOMA-IR, cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were adjusted for age, gender and BMI. ([#] Mean ± SD).

Logistic regression was also carried out to investigate the combined effect of the abovementioned risk alleles on the risk of becoming obese, as defined by Cole.[23] In order to investigate the combined effects of the risk alleles on the relative likelihood of obesity among the children participating in the study, the study sample was stratified into three groups: the children whose genotypes contained no risk alleles of the examined genes (reference group), the children whose genotypes contained 1-2 risk alleles and the children with genotypes of 3-4 risk alleles of MC4R and FTO. These genotypes were considered as the predictor variables in our logistic regression model, while the risk of becoming obese was considered our dependent variable. The level of significance was set at p <0.05. Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated.

RESULTS

The descriptive characteristics for both groups included are presented in Table 1. The mean age was approximately 11 years for both groups (p>0.05) and the overweight/obese population had, as expected, an elevated BMI, BMI-z-score, weight and weight z-score, waist circumference, waist-to-hip ratio, waist-to-height-ratio (p<0.001). The overweight/obese population also had a high birth weight (p=0.006) and increased height z-score (p<0.001).

Regarding the metabolic parameters of the two groups, the overweight/obese participants had significantly increased plasma insulin and HOMA-IR, higher serum triglyceride levels and a lower serum HDL-Cholesterol (p<0.001), while their serum glucose levels, systolic and diastolic blood pressure did not differ from the controls.

MC4R polymorphisms and anthropometric and metabolic profile

The observed genotype frequencies for the rs17782313 polymorphism of the MC4R gene were in Hardy-Weinberg equilibrium for both normal-weight and obese participants (p-value >0.05). The minor allele frequency was 0.227 for all subjects: 0.188 and 0.256 for normal-weight and overweight/obese subjects, respectively (data not shown).

Associations of MC4R rs17782313 genotypes with basic anthropometric indices are shown in Table 2. The rs 17782313 C risk allele schowed a significant association with BMI (p-value=0.01). Since there was no association to the height (p=0.89) the significant association to the BMI was through its impact on body weight (p=0.029). We also attempted to replicate the associations of the SNP MC4R rs17782313 with the risk of being obese (as defined for children and adolescents according to Cole[23]). The relative likelihood of obesity in children with the high-risk allele of rs17782313 SNP was 1.5 times higher than in normal weight subjects (p-value = 0.03).

Associations of the MC4R rs17782313 genotype with birth weight and markers of central adiposity (waist circumference and waist-hip ratio) were also investigated. However, no significant association was observed between the rs17782313 genotype and the birth weight or the indices of central adiposity. We further investigated whether the MC4R rs177823123 genotype was associated with metabolic parameters such as glucose or lipid metabolism indices; however, no significant association was observed.

FTO polymorphisms and anthropometric and metabolic profile

The observed genotype frequencies for rs9939609 of the FTO gene were in Hardy-Weinberg equilibrium for both normal-weight and obese subjects (p-value >0.05). The minor allele frequency was 0.440 for all subjects: 0.401 and 0.479 for normal-weight and obese subjects, respectively (data not shown).

Associations of FTO rs9939609 genotypes with basic anthropometric indices are shown in Table 2. The rs9939609 A risk allele showed significant association with BMI (p-value = 0.013), this effect apparently coming about through its impact on body weight (p-value = 0.011). Interestingly, in the case of FTO a significant association with height is observed (p-value = 0.040). We also attempted to replicate the association of the FTO SNP with the risk of being obese (as defined for children and adolescents according to Cole et al[23]). The relative likelihood of obesity in children with the high risk allele of the rs9939609 SNP was 1.4 times higher than for normal weight (p-value = 0.047).

As in the case of MC4R, associations of the FTO rs9939609 genotype with birth weight, markers of central adiposity (waist circumference and waist-hip ratio), glucose metabolism indices (glucose, insulin and HOMA-IR) and serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) were also tested. Waist circumference, waist to height and waist-to-hip ratios were all increased in children with the FTO risk allele (p-value = 0.01, 0.02 and 0.016, respectively) (Table 2). However, no association was found between the polymorphism and either glucose or lipid metabolism abnormalities.

Combined effect of the high risk alleles on the obese phenotype

We also applied logistic regression analysis in order to study the impact of combined risk alleles on the formation of an obese phenotype. Although the odds ratio of becoming obese was slightly elevated among children having one or two risk alleles (OR 1.059, 95% CI 0.587 - 1.910, p=0.85), for those bearing three or four risk alleles, the OR was more than four times increased when compared with the control group (OR 4.471, 95% CI 1.750 - 11.424 p=0.002), indicating a synergistic effect.

DISCUSSION

In the present study we investigated the impact of the presence of the risk alleles of two polymorphisms, one near the MC4R gene (rs17782313) that replaces

a threonine amino acid with cysteine (T->C) and one intrinsic to the FTO gene (rs9939609) that replaces a threonine amino acid with alanine (T->A) and their combined effect on childhood obesity. Both of them were significantly associated with the obese phenotype in Greek children and adolescents. Furthermore, both SNP risk alleles appear to affect weight and weight z-score ($p<0.05$), implying that their effect on BMI is mainly through gaining weight. The combined presence of three or more high risk alleles of both the FTO and MC4R genes confers a 4-fold higher risk for obesity in children and adolescents of Greek origin. Therefore, the combined effect of FTO and MC4R risk alleles was positively associated with an even higher risk for obesity, which is in accordance with findings of larger studies both in children and adolescents.[4,27-29]

A common finding of large-scale studies is that genetic variations of MC4R and FTO have their maximum effect during childhood that improves during adult life.[17,22,30,31] Elks et al in a recent longitudinal study found a positive effect of FTO and MC4R risk alleles on adiposity traits (BMI, weight) but also a positive effect on height during pre-adolescence, that attenuates with age.[27] The authors suggest that the age of 11 years, which is the mean age of our sample, is a landmark for development and that obesity affects growth tempo, making over-weight/obese children taller up to pre-adolescence. They further report that during the transition from adolescence to early adulthood adiposity no longer contributes to a taller stature.[27] Sovio et al[22] have reported a positive association between the rs9939609 A allele and BMI after the age of 5.5 years. They also report that this particular SNP accelerates the developmental age by 2.4% per risk allele, corresponding to an early adiposity rebound and eventually early puberty.[32] Haworth et al state that the rs9939609 A polymorphism progressively becomes more strongly associated with BMI after the age of 7.[33] Specifically in the case of twins, they report that heritability for BMI increased from 48% at the age of 4 years to 78% at the age of 11 years. Their results are consistent with the findings of previous studies on the effects of adiposity on the childhood growth trajectories.[34] Contrary to results presented by Elks et al, the rs9939609 A allele had no effect

on height in our cohort. In the present study we observed that the FTO risk allele was associated with height in the obese population (p=0.040), and this effect was attenuated after adjustment for age and sex (p=0.059), maintaining a positive trend that replicates the theory that this is the age when genetic predisposition typically affects growth.

Moreover, even though our obese group had increased birth weight (p=0.006), there was no correlation of this index to either the FTO or the MC4R risk alleles. This finding is in accordance with other studies.[17,30,32,35,36] Elks et al report a marginal positive correlation of birth weight to the obesity-risk-allele-score, but the correlation was significantly more robust after the first 6 weeks of life.[37] The FTO and MC4R risk alleles are thought to contribute to BMI increase through regulation of central nervous system messages to the hypothalamus, regulating appetite, satiety and calorie-dense preferences. Birth weight is strongly influenced by exogenous, mainly maternal, choices in the quantity and quality of provided energy, an effect that partly explains why the genetic risk alleles that modulate eating behavior have a minimal effect on weight. It could also imply that our obese population was born in a strongly "obesogenic" environment that has affected their prenatal development.

No association was found between the FTO and/or MC4R risk alleles and metabolic traits in our population. However, the obese/overweight subgroup already had insulin resistance and a dyslipidemic profile (low HDL-cholesterol and higher serum triglyceride levels), findings that are strongly associated with adiposity in both adults and children.[38] Our obese subpopulation did not exhibit high blood pressure, although previously, Xi Bo et al reported a positive association of the FTO risk allele with hypertension among obese homozygotes.[29] Their population extended to 18 years of age though, whereas our population is younger and has possibly been exposed for less time to the atherogenic effects of adiposity.

In conclusion, we found that the high risk alleles rs17782313 SNP of the MC4R gene and rs9939609 of the FTO gene had a significant impact on the occurrence of the obese phenotype in Greek children and adolescents. We did not, however, observe a

direct association between these high-risk alleles and the metabolic profile of the enrolled children, even though our obese subgroup already presented anthropometric and metabolic traits of central adiposity (waist-to-hip ratio, insulin resistance, low HDL, increased triglyceride levels).

The present study in the paediatric Greek population confirms the findings of similar large-scale studies conducted in other populations. It seems that the FTO and MC4R genes affect BMI and weight through modifications in eating behavior and habits, appetite and satiety, and not via alterations in energy expenditure. Pre-adolescence is the best time to study genetic influences, because eating behavior becomes less influenced by the family environment as the individual grows and forms his/her own tastes and habits. In early childhood, the youngster is highly family-dependent concerning the quality, quantity and schedule of meals, while during the transition to early adulthood eating habits are largely determined by social, cultural and psychological influences.[39] Earlier studies have demonstrated that lifestyle, physical activity and/or sedentary habits influence the expression of FTO and MC4R both in children and in adults.[40-42]

As the need for new treatment strategies to combat obesity becomes mandatory, more studies need to be carried out to elucidate the role of the MC4R and FTO polymorphisms in obesity and to determine whether they are associated with the development of lipid and glucose abnormalities as these children grow into adults. The obesity risk alleles have additive effects on the occurrence of the obese phenotype, but they can also be influenced and attenuated by lifestyle modification. This is why proper genetic screening of susceptible individuals at a preschool age may contribute to the establishment of healthy lifestyle habits, thereby alleviating the deleterious effects of persisting adiposity.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

FUNDING

None for all authors.

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