Research Communication

Obesity-Related Genetic Polymorphisms and Adiposity Indices in a Young Italian Population

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Abstract

Pediatric obesity develops when a complex biological predisposition collides with an obesogenic environment. To further elucidate the role of genetics in obesity onset, we performed a candidate-gene association study in a young and sportive Italian population by testing the association of functional polymorphisms in *ACE* (rs4646994), *FTO* (rs9939609), *MC4R* (rs17782313) and *PPARG* (rs1801282) genes with body mass index (BMI) and waist-to-height ratio (WHtR). We also tested the combinations of identified risk genotypes and epistatic interactions among them to determine the existence of cumulative effects in predicting the predisposition to gain weight. Our results confirm a significant direct influence of *MC4R* rs17782313 and *PPARG* rs1801282 on body composition, that is, minor allele homozygotes showed significantly higher BMI (rs17782313, $\beta = 1.258$, $P = 0.031$; rs1801282, $\beta = 6.689$, $P = 1.2 \times 10^{-4}$) and WHtR (rs17782313, $\beta = 0.021$, $P = 0.005$; rs1801282, $\beta = 0.069$, $P = 0.003$) values. Moreover, by leveraging multifactor dimensionality reduction and general linear model (GLM) approaches we identified an epistatic interaction between *ACE* and *MC4R,* where heterozygosity at *ACE* rs4646994 seems to protect from the unfavorable predisposition to gain weight given by C/C genotype at *MC4R* rs17782313 (GLM, $P = 0.004$). In conclusion, to clarify the role of genetics in multifactorial diseases remains a difficult goal, even for the most investigated polymorphisms and in controlled populations. Further studies on epistasis and gene–gene interaction will help to elucidate this complex scenario.

Keywords: *nutrigenetics; obesity; polymorphisms; PPARG; ACE; MC4R; FTO*

Additional Supporting Information may be found in the online version of this article.

Abbreviations: *ACE, angiotensin converting enzyme gene; AIC, Akaike information criterion; ASUR, Azienda Sanitaria Unica Regionale; BIC, Bayesian information criterion; BMI, body mass index; FTO, fat-mass and obesity-associated gene; I/D, insertion/deletion; IG, information gain; IOTF, International Obesity Task Force; MAF, minor allele frequency; MC4R, Melanocortin-4 receptor gene; MDR, multifactor dimensionality reduction; OECD, Organization for Economic Co-operation and Development; PCR, polymerase chain reaction; PPARG, peroxisome proliferator-activated receptor gamma; WHtR, waist-to-height ratio; MET, metabolic equivalents of task*

Volume 00, Number 00, Month 2017, Pages 00–00

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Received 15 November 2016; Accepted 12 December 2016
DOI 10.1002/iub.1596
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com)

Introduction

Pediatric obesity is one of the major public health problems in developed countries (1). Obese children have an increased risk of adult obesity (2) and a consequent lower quality of life due to greater risk of metabolic disorders and cardiovascular diseases (3). Pediatric obesity is rarely caused by a single-gene inherited defect. It generally develops in the presence of a complex biological predisposition becoming manifest when environmental and social conditions are characterized by obesogenic behaviors (4).

Body mass index (BMI) is the most commonly used quantitative measure of adiposity. The International Obesity Task Force (IOTF) sets that overweight is indicated by a BMI $\geq$85th percentile, whereas obese condition is determined by a BMI $\geq$95th percentile (5). Waist circumference (WC) represents another important index of excessive fat accumulation, particularly as indicator of abdominal fat, which has been widely associated with cardiovascular diseases (6). The waist-to-height ratio (WHtR) has also been suggested as a good predictor of abdominal obesity and metabolic risk factors (7,8). It may be applied to both sexes and ages, with a WHtR cut-off $\geq$ 0.50 defined as excess of abdominal fat (7,8).

The contribution of genetic components to the interindividual variation in common heritability of human adiposity has been estimated to range between 40% and 70% (4,9). However, the heritability of traits such as BMI, adiposity or obesity remains a matter of debate in pediatric obesity, even if the role of genetics has been unambiguously recognized (4,10,11). Several studies suggest that both the biology of fat storage and the variations in appetite and satiety regulation could represent important pathways implicated in obesity onset (12). The collective evidence highlights the difficulty of identifying major genes strictly related to common obesity because of the phenotypic complexity and the large numbers of genes involved, each of which exerts a small effect on the final phenotype (11,13).

Among the several candidate genes proposed so far, some have become very popular in the nutrigenetic field. In particular, genome-wide association studies (GWAS) identified *fat-mass and obesity-associated* gene (*FTO*, OMIM*610966, on chr. 16q12.2) as one of the most importantly related to obesity. *FTO* plays crucial roles in nucleic acid demethylation, management of energy homeostasis and regulation of body fat masses by lipolysis (14,15). Other studies also suggest that *FTO* may influence adiposity by affecting appetite (16). Interestingly, the most studied *FTO* variant, rs9939609, has been associated with obesity both in children and adults (17). The *melanocortin-4 receptor* gene (*MC4R*, OMIM*155541, on chr. 18q21.32) has been recently associated to overweight. Similar to *FTO*, *MC4R* regulates feeding control and energy balance (15,18). Rare *MC4R* loss-of-function mutations can cause monogenic forms of obesity (19), while the common variant rs17782313 has been consistently associated with obesity in European adults and children (19,20) and, notably, it showed a synergistic effect with *FTO* on the obese phenotype (21). Another widely studied gene is the *Peroxisome Proliferator-Activated Receptor Gamma* (*PPARG,* OMIM*601487, on chr. 3p25.2) (22). PPARG regulates adipocyte differentiation and influences BMI as well as glucose metabolism (23). A common nonsynonymous polymorphism (rs1801282, Pro12Ala) occurring in the second *PPARG* isoform, known as *PPARG2*, has been associated with higher BMI values, increased risk of obesity, cardiovascular diseases, type II diabetes and insulin resistance (24,25). The involvement of the Renin Angiotensin System (RAAS) has also been suggested in the pathophysiology of obesity, besides its well-known role in the onset of hypertension (26). Within the RAAS system, the role of Alu insertion-deletion (I/D) polymorphism (rs4646994) located on the *Angiotensin Converting Enzyme* gene (*ACE*, OMIM*106180, on chr. 17q23.3) has been extensively investigated. The presence of the deleted variant has been associated with higher levels of circulating ACE enzyme (27,28). *ACE* rs4646994 has also been linked to adiposity and metabolic outcomes (28,29). Interestingly, it has been shown that PPARs modulate RAAS through the transcriptional control of renin, angiotensinogen, ACE and angiotensin II receptor 1 (30), thus representing a possible functional link between *PPARG* and *ACE* genes.

Considering the potential of genetic information in nutritional surveillance and personalized nutrition, replication studies of polymorphisms widely inflated in the nutrigenetic field are needed. Moreover, it is known that genetic variants do not act singularly. Genes impact on the phenotype is modulated not only by external environment but also by other genes themselves (internal environment). Therefore, epistatic phenomena, in terms of both biological epistasis and statistical epistasis, should be clarified (31). To obtain a clearer picture of these aspects, at least for the most studied genes, it would be useful to identify the genetic makeup predisposing a subject to a certain disease. This is especially true for complex diseases where the role of genetics needs to be better defined.

So far, a limited number of studies focused on a selected group of candidate genes which could have a strong influence in the onset of obesity. Restricting the analysis to few candidate genes is a way to increase the power of studies with relatively small sample size. Moreover, the selection of a homogeneous population with a reduced number of confounding variables (participants with similar age, life style and geographical origin) should increase the effect size of a different genetic predisposition on weight gain.

Here, we conducted a study on a population of 306 Italian children and adolescents, who regularly play sports, with the aim of replicating and extending previous findings on *FTO* rs9939609, *MC4R* rs17782313, *PPARG* rs1801282 and *ACE* rs4646994 polymorphisms. Considering previous evidence on the possible cumulative effect of some of the mentioned polymorphisms (21,32,33), we also tested the existence of epistatic phenomena and the cumulative risk conferred by the combinations of these polymorphisms.

Experimental

Sample and Anthropometric Measurements

The study population was composed of 306 Italian healthy children and adolescents (173 males and 133 females), 7–18 years old (mean age 12.8 ± 2.5), recruited during a sport medicine check-up for the assessment of their health status to practice physical activity. All the participants regularly play sports, at least twice a week. According to CDC (Centers for Disease Control and Prevention, USA) guidelines (34), the level of physical activity of the studied population is estimated to be moderate (3–6 metabolic equivalents of task, METs). Subjects were recruited on a voluntary basis at Hospital ASUR of Area Vasta n°2-Jesi, Ancona, Italy, and parental consent was obtained from all participants, while the procedures were also explained to the child participants. The study protocol has been approved by the general direction of Area Vasta 2, Jesi, Marche (Italy), in accordance with the Declaration of Helsinki in its revised edition and with international and local regulatory requirements. Exclusion criteria were (a) current or previous occurrence of metabolic or digestive disease (except for appendectomy) or kidney disease; (b) being pregnant or breastfeeding. All the subjects were measured for height (bare feet maximum standing height with head horizontal and positioned with a Frankfurt plane), weight (class III scale with altimeter, Wunder A150) and WC (Hoechst mass roll fix tape) by trained evaluators according to standard protocols. Body mass index (BMI) was calculated as weight divided by height squared. Individuals with BMI percentile $\geq 85°$ according to sex and age were classified as overweight according to the IOTF cut-offs (5). The WHtR was determined by dividing the waist circumference of the enrolled subjects for their height, both measured in the same units.

Genotyping

Genomic DNA was extracted from buccal swabs through isopropanol precipitation. *FTO* rs9939609, *MC4R* rs17782313 and *PPARG* rs1801282 genotyping was performed using commercially available TaqMan SNP genotyping assays (Applied Biosystems, CA) on CFX-96 Biorad Real-Time PCR (Biorad, CA) according to the manufacturer's protocol. *ACE* rs4646994 was determined by polymerase chain reaction (PCR), as described by McCauley et al. (35).

Statistical Analysis

Statistical analysis was performed using the SPSS package for Windows, v.20.0 (SPSS Inc, Chicago, IL) and SNPStats (36) software. Genotypic, allelic frequencies and Hardy-Weinberg equilibrium (HWE) were also calculated. To test the proportion of variation in the quantitative variables (BMI, WHtR) explained by each polymorphism, linear regression models adjusted for age and sex were applied under codominant, dominant, recessive and over-dominant models. The best fitting model of association was determined using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) provided by SNPStats. The model with the lowest AIC and BIC values was considered the best fitting model.

Once the risk genotypes influencing body composition were identified, logistic regression was used to examine their cumulative effect under recessive model. A genetic predisposition score was calculated for each subject by summing the number of BMI (or WHtR)-increasing genotypes that were singularly associated to the phenotype.

To detect and characterize epistatic SNP–SNP interactions, a nonparametric and genetic model-free data mining method called multifactor dimensionality reduction (MDR) (37) was applied. MDR is a data reduction approach to identify multilocus genotype combinations that are associated with high or low risk of disease. Entropy-based interaction graphs were used as a visual tool for interpreting epistasis models (38,39). Gene–gene interaction test by general linear model (GLM) analysis was also performed to clarify the role of genetic polymorphisms showing a synergistic effect in MDR analysis. Throughout the study, the level of statistical significance was defined by a two-tailed *P*-value <0.05, except for GLM gene–gene interaction analysis where Bonferroni's correction was applied.

Results

Demographics

Among all the 306 enrolled subjects, 28% ($n = 85$) had a BMI $\geq$85th percentile ($n = 48$ male; $n = 37$ female) and 9% ($n = 30$) had a WHtR ≥ 0.5 ($n = 18$ male; $n = 12$ female).

Data collected by the Organization for Economic Co-operation and Development (OECD) about obesity in young European populations underline the emergent role of this problem in Italy. In fact, while obesity rates among adult Italians are lower compared to most OECD countries, the picture is definitely different for children: 36% of boys and 34% of girls are overweight or obese, compared to 23% of boys and 21% of girls, on average, in OECD countries (40). We can confirm that our population, composed of sportive children and adolescents, is relatively healthier than the general Italian population in terms of overweight. Considering the slightly lower prevalence of overweight among the analyzed subjects, the preventive role of practicing regular physical activity is confirmed.

Supporting Information Table 1 reports data relative to weight, height, BMI, WC and WHtR in the whole study population and according to sex. As expected, significant differences for weight, height and WC were detected between males and females, with males showing higher average values compared to females. On the contrary, the parameters normalized for height (such as BMI and WHtR) showed no significant differences.

Polymorphism Association Testing: Direct Effects

Genotype and minor allele frequencies of the four selected polymorphisms are reported in Table 1. All the polymorphisms were in HWE ($P > 0.05$) and minor allelic frequencies at *FTO* rs9939609, *MC4R* rs17782313 and *PPARG* rs1801282 were consistent with 1000 Genomes Project (41) Toscani population data. Allele frequency of *ACE* rs4646994 is not available

TABLE 1 *Genotypic and minor allele frequencies of the polymorphisms analyzed*

	ACE *rs4646994* N *(%)*		FTO *rs9939609* N *(%)*		MC4R *rs17782313* N *(%)*		PPARG2 *rs1801282* N *(%)*	
Genotype frequency	D/D	125 (0.41)	T/T	101 (0.33)	T/T	166 (0.54)	C/C	262 (0.86)
	I/D	135 (0.44)	A/T	144 (0.47)	C/T	110 (0.36)	C/G	41 (0.13)
	I/I	46 (0.15)	A/A	61 (0.20)	C/C	30 (0.10)	G/G	3 (0.01)
HWE P	0.330		0.490		0.086		0.400	
MAF	0.37		0.43		0.28		0.08	
MAF 1KG Toscani population	N/A		0.463		0.280		0.08	

HWE: Hardy–Weinberg equilibrium; MAF: minor allele frequencies.

through the 1000 Genomes Project (41), but the minor allele frequency is comparable to the one reported by Passaro et al. (33) in an Italian population (MAF = 0.38). Recessive model was considered as the best fitting for all the analyzed polymorphisms because of the lowest AIC and BIC values in comparison with the other models (Supporting Information Table 2).

Table 2 displays the association of each polymorphism with response variables (BMI and WHtR). Both *MC4R* and *PPARG* were significantly associated with higher BMI (rs17782313, $\beta = 1.258$, $P = 0.031$; rs1801282, $\beta = 6.689$, $P = 1.2 \times 10^{-4}$) and WHtR (rs17782313, $\beta = 0.021$, $P = 0.005$; rs1801282, $\beta = 0.069$, $P = 0.003$) values, while no association was found for *ACE* rs4646994 and *FTO* rs9939609.

MC4R and *PPARG* showed a cumulative risk both for BMI (OR: 1.508 (1.036–2.194); $P = 0.032$) and WHtR (OR: 2.167 (1.393–3.372); $P = 0.001$). Considering this genetic predisposition score, it becomes clear that the absence of homozygosis for the minor allele at both *MC4R* rs17782313 and *PPARG* rs1801282 loci represents a protective factor against body composition impairment.

Polymorphism Association Testing: Interacting Effects

As shown in Fig. 1, interaction links between *ACE* rs4646994 and *MC4R* rs1778231 are very synergic both in terms of BMI and WHtR. Indeed, a positive value of information gain (IG) indicates synergy between the two polymorphisms: in other words, the effect of the two genetic attributes is not linearly separable (42). However, the main effect, that is, the IG for the single genetic attribute, of *ACE* rs4646994 (0.99%) for WHtR was quite close to the pairwise interaction IG (1.02%); conversely for BMI, the main effect of *MC4R* rs17782313 (0.82%) was higher than the pairwise interaction IG (0.34%). The significance of main effects, even in presence of the synergic interaction *ACE* rs4646994 + *MC4R* rs17782313, is a particular condition, and we hypothesize that the relatively small sample size could be responsible for it. All the other pairwise interactions did not show evidence for synergic effects.

Thus, we performed a GLM analysis to better evaluate the interaction of *ACE* rs4646994 with *MC4R* rs17782313 (*ACE*MC4R*) on BMI and WHtR. *ACE*MC4R* showed a nominal association with BMI ($P = 0.038$). On the contrary, *ACE*MC4R* interaction was significantly associated with WHtR ($P = 0.004$). Thus, even if ACE does not exert a direct effect on body composition in our population, an interesting effect of its functional polymorphism has been showed: heterozygosity at *ACE* rs4646994 seems to protect *MC4R* rs17782313-C/C subjects from high WHtR levels (Fig. 2).

Discussion

Obesity is an important public health problem that increases morbidity and mortality, and it also represents a relevant economic burden for the healthcare system. Insufficient physical activity and excessive energy intake contribute to the increasing prevalence of obesity, but genetic factors strongly mediate the effect of the environment on each subject (4).

Significant associations between overweight, *MC4R* and *PPARG* polymorphisms were detected in our sample. To the best of our knowledge, their association with WHtR was never reported. A strong influence of *MC4R* rs17782313 on both body size and fat distribution was clearly observable in our population. The C/C genotype was significantly more frequent among individuals with higher BMI and WHtR values. This confirms the relationship between *MC4R* rs17782313 and obesity risk reviewed by Xi et al. (20) and evidenced by Lazopoulou in a European population of children (21). Despite the low MAF at *PPARG* rs1801282 and the previous contradictory results about its relationship with obesity (32), the *PPARG* G/G genotype resulted to be significantly associated with body overweight in our sample, confirming the results obtained in another young European population by Ochoa et al (43,44).

TABLE 2 *Association of genetic polymorphisms with BMI (A) and WHtR (B) under recessive model*

Gene	*rsID*	*Genotype*	N	*Response mean (S.E.)*	β *(S.E.)*	P
Polymorphisms association with BMI ($N = 306$, adjusted by age + sex)						
ACE	rs4646994	D/D–I/D	260	20.22 (0.21)	Ref.	
		I/I	46	20.63 (0.65)	0.629 (0.486)	0.197
FTO	rs9939609	T/T–A/T	245	20.16 (0.22)	Ref.	
		A/A	61	20.76 (0.46)	0.437 (0.434)	0.316
MC4R	rs17782313	T/T–T/C	276	20.13 (0.2)	Ref.	
		C/C	30	21.61 (0.78)	**1.258 (0.580)**	**0.031**
PPARG	rs1801282	C/C–C/G	303	20.2 (0.19)	Ref.	
		G/G	3	27.66 (3.67)	**6.689 (1.719)**	**1.2×10^{-04}**
(B) Polymorphisms association with WHtR ($N = 306$, adjusted by age + sex)						
ACE	rs4646994	D/D–I/D	260	0.43 (0)	Ref.	
		I/I	46	0.44 (0.01)	0.010 (0.006)	0.098
FTO	rs9939609	T/T–A/T	245	0.43 (0)	Ref.	
		A/A	61	0.44 (0.01)	0.002 (0.006)	0.711
MC4R	rs17782313	T/T–T/C	276	0.43 (0)	Ref.	
		C/C	30	0.45 (0.01)	**0.021 (0.008)**	**0.005**
PPARG	rs1801282	C/C–C/G	303	0.43 (0)	Ref.	
		G/G	3	0.5 (0.03)	**0.069 (0.023)**	**0.003**

Ref.: reference group; S.E.: Standard Error.
Data are summarized by means, standard errors and mean differences respect to a reference category and P values of the differences. AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; S.E.: standard error

However, given the extremely small number of recessive genotypes at *PPARG* rs1801282 in our population, the association we detected between the recessive genotype at *PPARG* and adiposity indices will necessarily require a validation in a larger sample size.

The lack of association between *ACE* polymorphism and body composition in our population is somewhat consistent with the very contradictory results reported in the literature (45,46). Marginean et al. (47) found higher BMI among I/I individuals in a Romanian children population, while other studies conducted in children and adolescent populations associated the D/D genotype with unfavorable anthropometric parameters (29,48). Even if the relationship of ACE with body size is controversial, there is evidence supporting the association of *ACE* polymorphism with body composition and response to physical training (49). Thus, it is conceivable that the effect of *ACE* rs4646994 on body composition in our population of young sportive Italians may be overshadowed.

Although previous data strongly associated *FTO* rs9939609 to BMI (17), we failed to detect a significant association between this polymorphism and body measurement. However, according to the recent IDEFICS study, conducted in a large population of European children, *FTO* rs9939609 effect on childhood obesity is significantly modulated by physical activity (50). Thus, similar to *ACE* rs4646994, the unfavorable effect of *FTO* rs9939609 may be minimized by regular physical activity.

Beyond the effect exerted by these polymorphisms, individually, on body composition, we tested the existence of epistatic interactions that may occur among them. Even if a possible interaction among *ACE*, *MC4R*, *FTO* and *PPARG* genes can be inferred by GWASs, which supported their relation with obesity and overweight, previous investigations did not test the presence of synergistic effects using a targeted approach. However, it should be mentioned that several studies have been conducted on this topic; a longitudinal study from Bouwman et al. (51) reported that adult men carrying *FTO* rs9939609 T/T

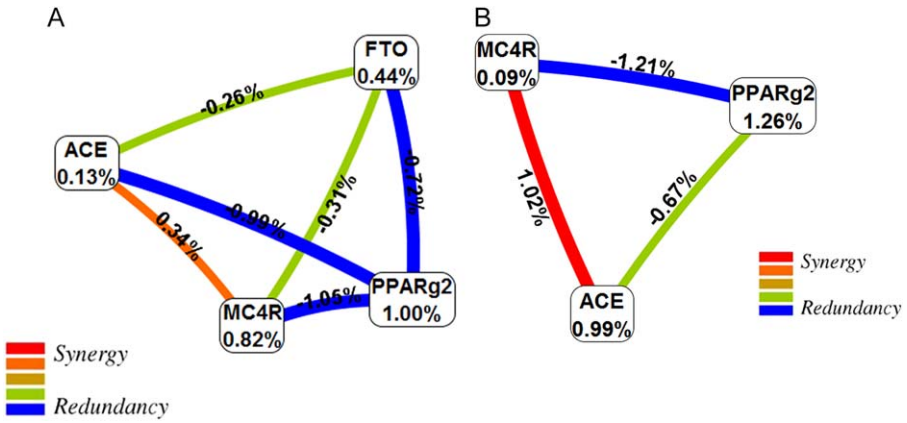

FIG 1 *Interaction maps with respect to the BMI (A) and to WHtR (B). In each map the nodes represent the genetic attributes, while the numeric values inside represent the main IG, that is, the effect of that specific genotype on the phenotypic class; the links are the interactions between the genetic attributes: in this case the numeric values show the IG of two genetic attributes. The scale color explains synergy or redundancy, that is, the effect cannot be linearly decomposed between the two genes involved. If the link color shifts to the blue, the two genes are redundant.*

genotype + *ACE* rs4646994-D allele were protected against weight gain over a 10-year period; Lazopoulou et al. (21) showed a cumulative effect of the presence of *FTO* rs9939609-A allele and *MC4R* rs17782313-C allele on childhood obesity in a Greek population; Goni et al. (52) showed that carrying unfavorable genotypes at both *FTO* rs9939609 and *MC4R* rs17782313 loci can influence weight loss in response to a hypocaloric diet; Carlos et al. (32) failed to prove the interaction of *FTO* rs9939609 and *PPARG* rs1801282 with obesity in Portuguese women; Passaro et al. (33) demonstrated a gene–gene interaction between *PPARG* rs1801282 and *ACE* rs4646994 on BMI and fat mass.

We found a cumulative effect on fat distribution exerted by the combination of *MC4R* rs17782313 and *PPARG* rs1801282. Besides the individual relation of these polymorphisms with increased BMI and WHtR, we found that the simultaneous absence of the risk genotype on both the two loci had a stronger protective effect against impairment of body composition.

Moreover, we examined *a priori* the effect of genetic attributes on phenotypic classes throughout the entropy-based IG analysis, that is, IG measures the epistatic interaction, or synergy, between SNPs on the phenotypic classes. According to the IG, it is possible to infer that the pairwise interaction *ACE* rs464699 + *MC4R* rs17782313 has a synergic effect for both BMI and WHtR, while all the other pairwise interactions are not synergic.

Thus, considering these data as preliminary evidence for a possible hidden interaction of these two genetic variants on the predisposition to gain weight, we carried out a gene–gene interaction analysis for the two gene variants with a synergic pairwise interaction. Results evidenced for the first time an interesting modulation exerted by *ACE rs4646994* on *MC4R* rs17782313 effect on WHtR phenotype. In particular, heterozygosis at *ACE rs4646994* can significantly protect individuals carrying the risk genotype at *MC4R* rs17782313 from higher WHtR values. A very recent study conducted by Kumari et al. (53) underlines a possible beneficial role of heterozygosis at ACE *rs4646994* locus in term of protection from cardiovascular diseases because of the reduced ACE levels that characterize heterozygous individuals compared to homozygous for both the alleles. Several theories have been discussed about the biological role of heterozygosis (54,55) and this is surely not the first case of an advantage conferred by that genotype.

Concluding, it is important to acknowledge the limitations of our study. The sample size and the number of functional genetic polymorphisms analyzed are relatively small. Nevertheless, the adopted selection criteria are sufficient to enhance the possible genetic influence on the phenotype. Furthermore, in support of the correlation between anthropometric parameters and genetic profile in the predisposition of overweight, recent evidence demonstrates that WHtR is a better indicator than BMI (56) for the early detection of obesity and obesity-related health risks in a population of sport active children.

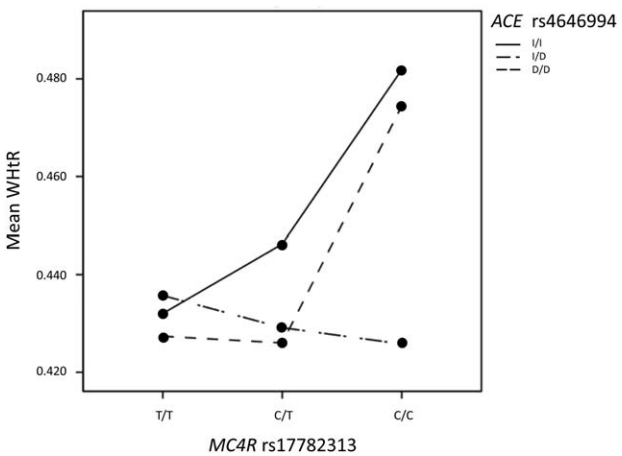

FIG 2 ACE *rs4646994 by* MC4R *rs17782313 interaction on WHtR. GLM analysis plot, using age and sex as covariates.*

Thus, correlating WHtR to genetic variants is a new and interesting hint. Accordingly, it is not surprising that the analyzed polymorphisms showed a stronger relation with WHtR, while no relevant findings were observed for BMI.

It is important to acknowledge that the huge amount of data generated by GWAS and other large genetic studies focused the attention on numerous polymorphisms that play a role on the predisposition toward obesity. However, replication of positive findings from genetic association studies helps ensure the validity that a genotype–phenotype association identified represents a reliable association and is not a chance finding or an artifact due to uncontrolled biases. In particular, we believe that an integrated approach not only describing the association of a single genetic variant with the phenotype tested but also dissecting its physiological role and the epistatic interactions with the other polymorphisms analyzed is necessary to really dissect the genetic predisposition for obesity risk.

Acknowledgment

The authors are grateful to all of the participants in the studies and to Dr. Emilio Amadio for his effort in the sampling procedure of the investigated population. They also thank Keyson s.r.l. to have cofinanced the doctoral project of the experimenter Laura Bordoni. Support for this research was entirely provided by professor Rosita Gabbianelli resources.

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