

Association study of common polymorphisms in *MSRA*, *TFAP2B*, *MC4R*, *NRXN3*, *PPARGC1A*, *TMEM18*, *SEC16B*, *HOXB5*, and *OLFM4* genes with obesity-related traits among Portuguese children

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Abstract

Background: At least 52 genetic *loci* were associated with obesity-related traits. However, little is known about the genetic basis of obesity among children. This study aims to test whether 10 polymorphisms in obesity-related genes *MSRA*, *TFAP2B*, *MC4R*, *NRXN3*, *PPARGC1A*, *TMEM18*, *SEC16B*, *HOXB5* and *OLFM4* are associated with the risk of obesity in Portuguese children.

Methods: A total of 730 children aging from 6 to 12 years-old, recruited randomly from public schools in Portugal, were analyzed. Anthropometric measurements were obtained and children were classified into three phenotypic groups, normal-weight (n=256), overweight (n=320), and obese (n=154), according to the IOTF cut-offs. Polymorphisms were genotyped by allelic discrimination TaqMan assays.

Results: The *MC4R* rs12970134 polymorphism was nominally associated with BMI ($p=0.035$), BMI Z-score ($p=0.043$) and waist circumference ($p=0.020$), and borderline associated with weight ($p=0.053$). Near nominally associations were also found for the *PPARGC1A* rs8192678 polymorphism with weight ($p=0.061$), and for the *MSRA* rs545854 polymorphism with BMI ($p=0.055$) and BMI Z-score ($p=0.056$). Furthermore, logistic regression showed that *MC4R* rs12970134 and *TFAP2B* rs987237 were nominally, respectively, associated ($p=0.029$) and borderline associated ($p=0.056$) with the obese phenotype.

Conclusion: This study highlighted the possible association of *MC4R*, *PPARGC1A*, *MSRA* and *TFAP2B* polymorphisms with several obesity-related traits in a sample of Portuguese children. The two significant associated *TFAP2B* rs987237 and *MC4R* rs12970134 polymorphisms showed an opposite direction of effect to that in the original reports.

Introduction

The obesity phenotype has been increasing in the last decades and the causes for this complex disorder are thought to be related with an imbalance between energy intake and energy expenditure due to changes in lifestyles including exposure to an obesogenic environment.¹ Furthermore, it is estimated that the heritable predisposition to obesity may range from 40 to 70%.²⁻⁴

In less than seven years, genome wide association studies (GWAS) have successfully identified more than 50 genetic *loci*, which were unequivocally associated with obesity-related traits.⁵ The first *locus* associated with obesity was the fat mass and obesity associated (*FTO*) gene by Frayling *et al.*,⁶ which is the most replicated gene across the world, both in adult and children samples,⁷⁻⁹ including in the Portuguese population.¹⁰ Subsequently, several other studies emerged associating single nucleotide polymorphisms in several genes across the genome, most of them in adults,^{11, 12} and a few in children.¹³ Nevertheless, candidate and replication studies of obesity *loci* among different populations emerge as an important step to identify and clarify which variants are indeed associated with obesity. The frequency of obesity-susceptibility alleles varies between populations, and these allele distributions could be a consequence of population-specific obesogenic environments associated with specific demography and cultural histories. Replication studies are also relevant to determine which polymorphisms previously associated with obesity in adults are also linked in children, and, in a final instance, to better understand the complexity of obesity susceptibility.

In this study, a sample of Portuguese children was tested for the association of obesity and obesity-related quantitative traits with ten polymorphisms in nine candidate genes including, methionine sulfoxide reductase A (*MSRA*), transcription factor AP-2 beta (*TFAP2B*), melanocortin 4 receptor (*MC4R*), neurexin 3 (*NRXN3*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*), transmembrane protein 18 (*TMEM18*), homolog of *S. cerevisiae* Sec16 (*SEC16B*), homeobox B5 (*HOXB5*) and olfactomedin 4 (*OLFM4*).

Material and Methods

Study subjects

The original sample consisted of 1433 Portuguese children of European descent, aging between 6-12 years old, randomly selected from several public schools in the central region of Portugal.¹⁴ From this original sample, three body mass index (BMI) groups, using age and sex specific BMI cut-offs provided by the International Obesity Task Force (IOTF),¹⁵ were attained in a total of 730 children, including: i) 154 obese subjects (resulting from the BMI in adult's cut-points ≥ 30 kg/m²); ii) 320 overweight subjects (resulting from the BMI in adult's cut-points between 25 kg/m² and 29 kg/m²); and iii) 256 lean controls randomly selected from the initial group of 959 children with BMI <25 kg/m².

The study protocol was approved by *Direção-Geral de Inovação e de Desenvolvimento Curricular*, the ethical Committee of the Portuguese Ministry of Education, and was conducted in accordance with the institutional and ethical guidelines of the University of Coimbra. Written informed consent was previously obtained from the children's parents.

Anthropometric measurements

All children underwent anthropometric measurements of height, weight, waist and hip circumference using a standardized protocol. Body weight (kg) and height (cm) were taken with participants dressed in lightweight clothing without shoes to determine the BMI. Waist circumference (WC) (cm) was measured midway between the lowest rib and the iliac crest, to the nearest 0.1 cm after inhalation and exhalation. Hip circumference (cm) was measured at the point over the buttocks yielding the maximum circumference. The BMI was calculated by dividing weight (in kilograms) by height (in meters) squared (kg/m²). Abdominal obesity was defined using the sex and age-specific ≥ 90 th waist circumference percentile.¹⁶

Selection and Genotyping of polymorphisms

Ten single nucleotide polymorphisms identified from the literature significantly associated to obesity or obesity-related traits in children of European origin were selected: rs17782313 and rs12970134 near *MC4R*, prominent in the literature; rs10146997 in *NRXN3*, rs8192678 in *PPARGC1A*, rs7561317 near *TMEM18* and rs10913469 in *SEC16B*, poorly studied; rs545854 in *MSRA* and rs987237 in *TFAP2B*, associated in adult's populations¹¹ but never replicated in children; and rs9299 in *HOXB5* and rs9568856 in *OLFM4*, recently associated with childhood obesity¹³ but never replicated.

The genomic DNA was extracted from buccal cells using the PureLink® Pro 96 Genomic DNA Kit (Invitrogen Corporation, Carlsbad, CA, USA), according to the instructions of the manufacturer.

Samples were genotyped for all SNPs by allelic discrimination assays using TaqMan probes (Applied Biosystems, Foster City, USA). All polymerase chain reactions (PCRs) were done in a volume of 20 µl containing in 1x SsoFast™ Probes Supermix (Bio-Rad, Hercules, CA, USA), 0.5 µl of specific TaqMan SNP Genotyping Assays (20x) (Applied Biosystems, Foster City, USA) and about 40 ng of genomic DNA, according to the manufacturer's instructions. Thermal cycling conditions were 10 min at 95°C, and 35 cycles each of 95°C for 15 sec and 60°C for 1 min. The fluorescence was observed through a MiniOpticon real time PCR system (Bio-Rad, Hercules, CA, USA). To assess genotyping reproducibility, 10% of random samples were selected and re-genotyped for all SNPs with 100% concordance.

Statistical analysis

The allelic and genotypic frequencies of all polymorphisms were estimated by direct counting. Hardy-Weinberg equilibrium probability values were achieved using an exact test.¹⁷ Logistic regression under an additive genetic model, allowing for analysis of a binary outcome (a case-control *status*), was used to test obesity and overweight phenotype polymorphism associations, adjusted for age and sex, by calculating odds ratios (OR) with 95% of confidence intervals (CI) and *p*-values. For *MC4R* rs17782313 and rs12970134 polymorphisms, linkage disequilibrium (r^2) values and a case/control (normal vs. obese) haplotype association were

assessed. All these statistical analyses were done by using the set-based tests implemented on PLINK software v.1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>).¹⁸ For each obesity-related quantitative parameters (BMI, BMI Z-score, weight and waist circumference), the nonparametric Kruskal-Wallis test was used to evaluate differences among the three genotypes in all the polymorphisms. Normality of the data was assessed using the Kolmogorov-Smirnov test. This statistical analysis was performed using the SPSS software (for windows, version 18.0). A significant *p*-value was considered below 0.005 (0.05/10) by applying a Bonferroni correction for multiple testing, and a *p*-value between 0.005 and 0.05 has been considered as nominally significant.

QUANTO, v.1.1 power calculator (<http://hydra.usc.edu/gxe/>) was used to estimate the power of association as a function of the frequency of the effect allele assuming an additive model.¹⁹

Results

The anthropometric characteristics of the study subjects distributed by phenotype are shown in Table 1.

The genotyping success rate varied between 96.3% and 99.7%. The minor allele frequencies observed for all polymorphisms in the total sample were: 15% for rs7561317-A (*TMEM18*), 16% for rs10913469-C (*SEC16B*), 36% for rs8192678-A (*PPARGC1A*), 18% for rs987237-G (*TFAP2B*), 16% for rs545854-C (*MSRA*), 12% for rs9568856-A (*OLFM4*), 18% for rs10146997-G (*NRXN3*), 32% for rs9299-C (*HOXB5*), 21% for 17782313-C (*MC4R*) and 22% for rs12970134-A (*MC4R*) (Table 2). These frequencies are in accordance with those found in the HapMap CEU population (<http://www.ensembl.org/>). Genotype distributions among the control group were in agreement with Hardy-Weinberg equilibrium for all the studied polymorphisms ($p > 0.05$).

We analysed the obesity-related quantitative traits BMI, BMI Z-score, weight and waist circumference among different genotypes for each of the studied polymorphisms. The mean

values for the anthropometric traits among the three different genotypes are detailed in Table 2. The *MC4R* rs12970134 major G-allele was found nominally associated with increase in BMI ($p=0.035$), BMI Z-score ($p=0.043$) and waist circumference ($p=0.020$), and borderline associated with weight ($p=0.053$). Near nominal associations were also found for the *PPARGCIA* rs8192678 minor A-allele with weight ($p=0.061$) and waist circumference ($p=0.093$), and for the *MSRA* rs545854 major G-allele with BMI ($p=0.055$) and BMI Z-score ($p=0.056$). After correction for multiple testing no statistically significant associations were found.

Genotype distributions of obesity-related parameters for the *MC4R* rs12970134 polymorphism, which showed the highest statistical significant associations with the obesity-related traits, are detailed in Figure 1. Homozygotes for the minor A-allele have the lowest value distributions for all the analysed quantitative parameters (BMI, BMI Z-score, waist circumference and weight).

Logistic regression analysis, in an additive model, revealed that *MC4R* rs12970134 major G-allele was nominally associated with the obesity risk (OR = 1.477; $p=0.029$) and that *TFAP2B* rs987237 major A-allele and *TMEM18* rs7561317 major G-allele are near nominally associated with the risk of obesity (OR = 1.455; $p=0.056$; and OR = 1.416; $p=0.092$, respectively) (Table 3). Only the *PPARGCIA* rs8192678 polymorphism was found nominally associated with the overweight phenotype (OR = 1.297; $p=0.041$) (Table 3).

We further investigated the difference in the genotype distribution between cases and controls for abdominal obesity by logistic regression analysis. In the total of 730 children, 112 revealed abdominal obesity (using sex and age-specific $\geq 90^{\text{th}}$ waist circumference percentile). From the ten polymorphisms studied, only *TMEM18* rs7561317 (major G allele), showed nominal association with increased abdominal obesity (OR = 1.589; 95% CI, 1.02-2.50; $p=0.042$).

The haplotype analysis for the two *MC4R* rs17782313 and rs12970134 polymorphisms, located at a distance of 33.5 Kb and in high linkage disequilibrium ($r^2 = 0.74$), revealed that the

common TG haplotype was associated with the risk of obesity ($p=0.043$) (with frequency of 81.2% in the obese group *versus* 75.0% in the control group).

Discussion

Understanding the genetic basis of obesity in children could be used as a first step to develop possible preventive measures. Recent GWAS have identified many (more than 50) different genetic variants conferring susceptibility to obesity.²⁰ However, the modest association with the obesity risk observed for most variants implies that replication studies in different populations are required to detect and confirm such signals of association, eliminating false positives that may arise by chance or systematic bias.

Focused on ten polymorphisms across the genome (located in or near the *MSRA*, *TFAP2B*, *NRXN3*, *PPARGC1A*, *TMEM18*, *SEC16B*, *HOXB5*, *OLFM4* and *MC4R* genes), previously associated by GWAS with obesity-related outcomes in populations of European origin, we conducted a genetic association study to investigate their role in the susceptibility of obesity in a sample of Portuguese children. Using obesity-related quantitative traits to assess whether the genotypes predicts the trait value, we identified the *MC4R* rs12970134 *loci* nominally associated with several obesity-related traits, and two *loci* (*PPARGC1A* and *MSRA*) near nominally associated with at least one anthropometric parameter (Table 2). In addition, using logistic regression analyses, the *MC4R* rs12970134 polymorphism was found nominally associated ($p=0.029$) with the risk of obesity and the *TFAP2B* rs987237 polymorphism showed borderline significant association ($p=0.056$) with the obese phenotype. Thus, this study highlights these four polymorphisms as potential genetic markers of the obesity phenotype in this Portuguese children sample.

The *MC4R* gene is known to be the most common cause of monogenic obesity in extreme childhood obesity,⁵ but also its flanking genomic region has been the third strongest implicated in polygenic obesity. The expression of *MC4R* is restricted to the hypothalamus involved in food intake regulation.²¹ Until now, several variations of this gene were established with BMI

and/or waist circumference, showing an independent role in body variation. Polymorphisms rs12970134 and rs17782313, located 154 kb and 188 kb, respectively, downstream of the *MC4R* gene, were found associated with obesity and obesity-related traits in several studies in Asian and European populations, both in children and adults.²²⁻²⁷ In the present study, nominal significant associations were found between *MC4R* rs12970134 and BMI, BMI Z-score and waist circumference ($p < 0.05$), as also with the risk of obesity ($p = 0.029$). However, our findings do not replicate previous reports that show the minor A-allele associated with increased risk in BMI and waist circumference in children of European^{22, 24-26, 28} or Asian²⁷ descent. Instead, in the present study, Portuguese children showed the minor rs12970134-A allele significantly associated with lower BMI ($p = 0.035$), BMI Z-score ($p = 0.043$), waist circumference ($p = 0.020$), and also with a lower risk for the obesity phenotype (OR = 0.677; $p = 0.029$). For this polymorphism, the minor A-allele frequency was 18.5% in the obese group *versus* 25.1% in the control group. For the second *MC4R* polymorphism, rs17782313, previous studies showed the C-allele associated with childhood obesity (increasing BMI in $\pm 0.22 \text{ kg/m}^2$),²³ however, in the Portuguese children, no statistical association was found with any obesity-related trait. The haplotype analysis showed the *MC4R* rs17782313 / rs12970134 TG haplotype associated with the risk of obesity, confirming the potential role of rs12970134 major G-allele in the aetiology of obesity in our sample of Portuguese children.

A GWA scan meta-analysis conducted by Lindgren *et al.*²⁹ found that the G allele of the *MSRA* rs545854 polymorphism was associated with waist circumference ($p = 8.9 \times 10^{-9}$) in adults. Bille *et al.*¹¹ also found significant association between this polymorphism and waist circumference (OR = 1.08; $p = 0.02$). In our study, nominal borderline significant associations with BMI ($p = 0.055$) and BMI Z-score ($p = 0.056$) were observed. The biological function between *MSRA* locus and adiposity remain unclear.²⁹

PPARGC1A is a transcriptional co-activator that has been implicated in the regulation of genes involved in energy metabolism.³⁰ The Gly482Ser missense mutation (rs8192678), predicted by a G-to-A transition at position +1,564 in exon 8 of the *PPARGC1A* gene, was found associated with obesity indices in middle-aged women of a cross-sectional Austrian

population³⁰ and with abdominal obesity in Chinese adults.³¹ In our study, near nominal associations were obtained with weight ($p=0.061$) and waist circumference ($p=0.093$) for the 482Ser variant.

The molecular function of the *TMEM18* gene product is to bind DNA to suppress transcription; it could be differently expressed in the hypothalamus and is possibly involved in the regulation of feeding.³² Polymorphism rs7561317, located about 22kb downstream of *TMEM18*, is the second best associated *locus* with BMI after *FTO* gene.⁵ A GWA study conducted by Thorleifsson *et al.*³³ found that the rs7561317 GG genotype increases BMI in $\pm 0.70\text{kg/m}^2$ and is associated with obesity. In the present study, significant associations were not found between any obesity-related trait and the *TMEM18* rs7561317 polymorphism, however, our findings are directionally consistent with previous studies conducted in children and adolescents, as the rs7561317 major G-allele was found marginally associated with the risk of obesity (OR = 0.706; $p=0.092$).

The *TFAP2B* gene is suggested to be involved in global adipocyte response to positive energy balance.²⁹ The minor G-allele of rs987237 polymorphism was previously found associated with increased waist circumference ($p=1.9\times 10^{-11}$) and BMI ($p=7.0\times 10^{-12}$) in a meta-analysis of 16 GWAS within adults of European ancestry;²⁹ and also in children it was found associated with increased waist circumference ($p=3.5\times 10^{-2}$) and BMI ($p=0.06$).²⁸ Our data in Portuguese children do not replicate previous findings while we observed a nominal borderline significant association of the major rs987237 A-allele with the risk of obesity (OR = 0.692; $p=0.056$).

In the present study, the major *MC4R* rs12970134-G and *TFAP2B* rs987237-A alleles showed, respectively, nominal and near-nominal significant associations with the risk of obesity, but the direction of effect was reverse when comparing to that in the original reports. Despite opposite direction on the effect of a risk allele is highly unlikely, this was observed in several studies including between different populations³⁴ but also inside a same population.³⁵ For the *TFAP2B* rs987237 polymorphism, the original significant association was found in adults,²⁹ and to the authors knowledge, this study with Portuguese participants is the first

replication report involving children. Therefore, the opposite direction of association for rs987237-A risk allele could be due to differences between children and adults regarding natural physiological differences.

All genes studied in this work are considered as candidates for the risk of developing obesity. Most of them are involved in homeostasis and energy metabolism, nevertheless the casual effect of these polymorphisms in the pathogenesis of obesity remains unclear. In the present study with Portuguese children, only the *MC4R* rs12970134 polymorphism showed nominal significant association ($p < 0.05$) with obesity and most of obesity-related traits. In a previous study for the *FTO* gene using the same cohort of individuals,¹⁰ significant associations were also found between the rs9939609 minor A-allele and increased risk for several anthropometric traits, including weight ($p = 0.019$), BMI ($p = 0.018$), BMI Z-score ($p = 0.011$) and waist circumference ($p = 0.016$), in concordance with reports worldwide. Thus, both *loci FTO* and *MC4R* appear to play a key role in the obesity phenotype in Portuguese children. Most of the analysed polymorphisms in this present study showed no nominal effects in obesity-related traits, but this difference with the previous findings may be due partly to the sample size, which may have been insufficient to replicate the original findings. The estimated power of association observed ranges from 6% to 72%, but at least for *MC4R* rs12970134 polymorphism, the variant most associated to obesity in our sample, the obtained power (72%) is close to values ($\geq 80\%$) commonly considered sufficient to detect genetic variant interaction effects.

In conclusion, among the 10 *loci* reported in this study, polymorphisms in or near *MC4R*, *PPARGC1A*, *MSRA* and *TFAP2B* genes could be assumed to play a role in the risk of obesity in this population sample of Portuguese children. While we could not replicate the original findings concerning the direction effect of the *MC4R* rs12970134 and *TFAP2B* rs987237 risk alleles our results deserve confirmatory studies in other populations. Moreover, our data may show that the polymorphisms provided here could play a modest role in the obesity aetiology in children, at least when comparing with the *FTO* gene, suggesting the existence of others unknown *loci* involved in the obesity susceptibility. Our replication study could also have

public health significance while genes playing an essential role in energy homeostasis, such as *PPARGC1A* or *TFAP2B*, suggested by our data as obesity-related genes, may be used as targets for obesity treatment. Further investigations in the near future regarding genetic associations and functional roles of these polymorphisms should be helpful to confirm its implication in the development of obesity and if they could be attractive targets for therapeutic agents.

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Conflict of interests

The authors declare no conflict of interest.

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Legends:

Figure 1. Box plot demonstrating the distribution of untransformed BMI, BMI Z-score, weight and waist circumference within each genotype group of MC4R rs12970134 polymorphism. Each box represents the anthropometric traits values between the 25th and 75th quartiles, and the dark line within the boxes indicates the median values.

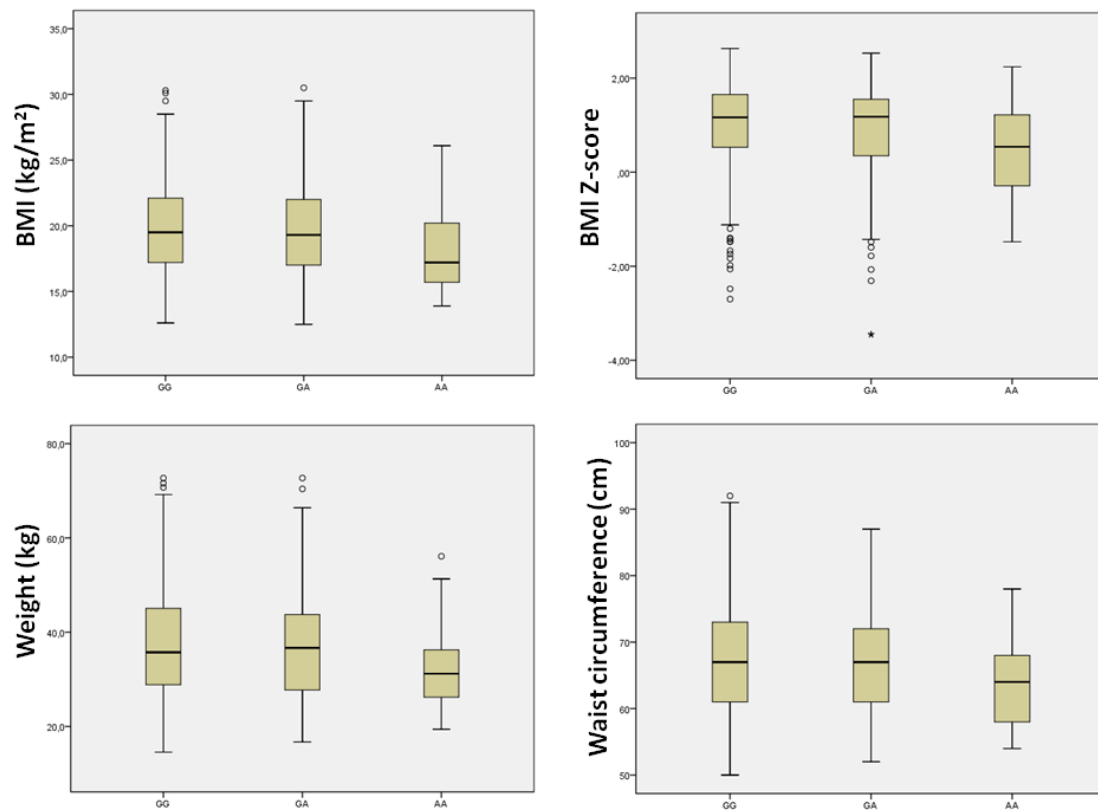


Table 1. General characteristics of the Portuguese children participants.

	Total	Phenotype distribution*		
		Normal	Overweight	Obese
Subjects	730	256	320	154
Girls, %	50.7	55.9	45.3	53.2
Age, years	9.1 ± 1.7	8.6 ± 1.6	9.5 ± 1.6	9.0 ± 1.7
Height (cm)	136.2 ± 11.7	131.1 ± 11.1	139.5 ± 11.1	137.9 ± 10.6
Weight (kg)	37.2 ± 11.3	28.1 ± 6.6	40.2 ± 9.3	46.1 ± 11.0
BMI (kg/m ²)	19.6 ± 3.4	16.1 ± 1.5	20.3 ± 1.8	23.8 ± 2.5
BMI Z-score	0.93 ± 0.97	-0.15 ± 0.78	1.3 ± 0.23	1.99 ± 0.23
WC (cm)	67.2 ± 7.8	60.3 ± 4.5	68.9 ± 5.4	75.1 ± 6.6
HC (cm)	79.0 ± 10.3	70.4 ± 6.5	81.9 ± 8.0	87.1 ± 9.4
WHR	0.85 ± 0.06	0.86 ± 0.06	0.85 ± 0.06	0.87 ± 0.05

Data are presented as mean ± standard deviation.

*Phenotype distribution was determined using age and sex specific BMI cut-offs provided by the International Obesity Task Force (IOTF).

Abbreviations: BMI, body mass index; BMI Z-score, body mass index standard deviation score; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.

Table 2. Minor allele frequencies and Hardy-Weinberg equilibrium test of the 10 studied polymorphisms in the sampled Portuguese children and their associations with obesity-related quantitative traits.

Polymorphism	Chr.	Gene	Alleles*	n	MAF	HWE	No. 11/12/22	Genotype (mean ± SD)			p-value
								11	12	22	
rs10913469	1q25	SEC16B	C:T	728	0.16	0.479	20/186/522				
BMI (kg/m ²)								19.6 ± 3.6	19.5 ± 3.5	19.6 ± 3.4	0.930
BMI Z-score								0.99 ± 0.9	0.92 ± 1.0	0.93 ± 1.0	0.902
Weight (kg)								37.5 ± 13.4	36.9 ± 11.9	37.0 ± 11.1	0.736
Waist C (cm)								67.9 ± 8.6	66.9 ± 8.3	67.3 ± 7.5	0.709
rs7561317	2p25	TMEM18	A:G	721	0.15	0.886	16/191/514				
BMI (kg/m ²)								19.2 ± 3.6	19.2 ± 3.3	19.7 ± 3.5	0.141
BMI Z-score								0.81 ± 1.1	0.84 ± 0.9	0.97 ± 1.0	0.120
Weight (kg)								36.7 ± 13.1	36.3 ± 11.4	37.6 ± 11.2	0.306
Waist C (cm)								66.1 ± 7.7	66.3 ± 7.5	67.6 ± 7.8	0.121
rs8192678	4p15	PPARGC1A	A:G	703	0.36	1.000	89/323/291				
BMI (kg/m ²)								20.0 ± 3.6	19.8 ± 3.4	19.4 ± 3.4	0.198
BMI Z-score								0.99 ± 1.0	0.97 ± 0.9	0.89 ± 1.0	0.359
Weight (kg)								38.9 ± 12.3	38.0 ± 11.3	36.1 ± 10.9	0.061
Waist C (cm)								68.9 ± 8.7	67.5 ± 7.6	66.6 ± 7.5	0.093
rs987237	6p12	TFAP2B	G:A	725	0.18	0.615	21/218/486				
BMI (kg/m ²)								19.0 ± 2.9	19.3 ± 3.2	19.7 ± 3.5	0.392
BMI Z-score								0.87 ± 1.0	0.89 ± 1.0	0.94 ± 1.0	0.536
Weight (kg)								34.1 ± 9.4	36.8 ± 11.2	37.5 ± 11.5	0.353
Waist C (cm)								64.9 ± 7.8	66.6 ± 7.3	67.6 ± 7.9	0.130
rs545854	8p23	MSRA	C:G	717	0.16	0.334	22/187/508				
BMI (kg/m ²)								18.3 ± 3.7	19.8 ± 3.6	19.7 ± 3.4	0.055

									0.51 ± 1.1	0.96 ± 1.1	0.95 ± 1.0	0.056
									34.3 ± 10.6	38.0 ± 11.7	37.1 ± 11.4	0.240
									65.1 ± 6.9	67.5 ± 8.3	67.3 ± 7.5	0.282
rs9568856	13q14	<i>OLFM4</i>	A:G	725	0.12	0.059	17/141/567					
									19.2 ± 3.6	19.9 ± 3.4	19.5 ± 3.4	0.374
									0.92 ± 0.8	0.97 ± 0.9	0.92 ± 1.0	0.719
									35.6 ± 12.2	38.1 ± 11.1	37.0 ± 11.4	0.286
									65.7 ± 8.4	68.0 ± 7.5	67.1 ± 7.8	0.248
rs10146997	14q31	<i>NRXN3</i>	G:A	716	0.18	0.798	21/212/483					
									19.1 ± 3.5	19.6 ± 3.3	19.7 ± 3.5	0.672
									0.91 ± 1.0	0.92 ± 1.0	0.95 ± 1.0	0.579
									34.3 ± 11.9	37.5 ± 11.3	37.4 ± 11.3	0.436
									64.6 ± 7.6	67.3 ± 7.7	67.5 ± 7.8	0.260
rs9299	17q21	<i>HOXB5</i>	C:T	727	0.32	0.672	78/313/336					
									19.5 ± 3.4	19.7 ± 3.6	19.5 ± 3.3	0.710
									0.87 ± 1.0	0.94 ± 1.0	0.92 ± 0.9	0.751
									37.3 ± 11.6	37.5 ± 11.3	37.0 ± 11.4	0.792
									66.9 ± 7.0	67.4 ± 7.9	67.2 ± 7.8	0.896
rs12970134	18q21	<i>MC4R</i>	A:G	729	0.22	0.163	29/266/434					
									18.1 ± 3.3	19.5 ± 3.5	19.7 ± 3.9	0.035
									0.53 ± 1.0	0.89 ± 1.0	0.98 ± 0.9	0.043
									32.5 ± 9.1	37.1 ± 11.3	37.7 ± 11.5	0.053
									63.6 ± 5.8	67.1 ± 7.6	67.6 ± 7.9	0.020
rs17782313	18q22	<i>MC4R</i>	C:T	716	0.21	0.432	28/247/441					
									18.6 ± 3.6	19.7 ± 3.4	19.6 ± 3.5	0.269
									0.61 ± 1.1	0.95 ± 1.0	0.94 ± 1.0	0.257
									34.4 ± 10.6	37.7 ± 11.0	37.2 ± 11.5	0.260
									65.6 ± 7.5	67.4 ± 7.3	67.3 ± 8.0	0.430

Abbreviations: Chr., chromosome; *n*, number of genotyped children; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; BMI, body mass

Index; BMI Z-score, body mass index standard deviation; WC, waist circumference; HC, hip circumference.

* Alleles: Minor (1): Major (2).

p-values were obtained using the Kruskal-Wallis test.

p-value nominally significant ($p < 0.05$) are in bold and italic.

Table 3. Allele frequencies of the 10 studied polymorphisms in the Portuguese children, and their associations with risk of obesity among phenotypic groups.

SNP ID/ Gene	Allele	Obese vs. normal				Overweight vs. normal		
		Normal*	Obese*	OR CI 95%	<i>p</i>	Overweight*	OR CI 95%	<i>p</i>
rs10913469 <i>SEC16B</i> T>C	T C	0.855 0.145	0.847 0.153	1.058 (0.71-1.57)	0.780	0.825 0.175	1.154 (0.83-1.59)	0.387
rs7561317 <i>TMEM18</i> G>A	G A	0.825 0.175	0.869 0.131	0.706 (0.47-1.06)	0.092	0.850 0.150	0.829 (0.60-1.13)	0.248
rs8192678 <i>PPARGC1A</i> G>A	G A	0.678 0.322	0.642 0.358	1.176 (0.86-1.59)	0.293	0.619 0.381	1.297 (1.00-1.66)	0.041
rs987237 <i>TFAP2B</i> A>G	A G	0.804 0.196	0.856 0.144	0.687 (0.46-1.01)	0.056	0.817 0.183	0.915 (0.67-1.23)	0.559
rs545854 <i>MSRA</i> G>C	G C	0.820 0.180	0.836 0.164	0.960 (0.65-1.41)	0.838	0.847 0.153	0.878 (0.63-1.20)	0.425
rs9568856 <i>OLFM4</i> G>A	G A	0.882 0.118	0.866 0.134	1.156 (0.75-1.77)	0.504	0.884 0.116	0.985 (0.68-1.41)	0.935
rs10146997	A	0.818	0.860	0.731 (0.49-1.08)	0.119	0.808	1.07 (0.79-1.45)	0.645

<i>NRXN3</i> A>G	G	0.182	0.140			0.192		
rs9299	T	0.679	0.654	1.122 (0.83-1.51)	0.453	0.687	0.962 (0.74-1.23)	0.762
<i>HOXB5</i> T>C	C	0.321	0.346			0.312		
rs12970134	G	0.749	0.815	0.677 (0.47-0.96)	0.029	0.782	0.829 (0.63-1.09)	0.183
<i>MC4R</i> G>A	A	0.251	0.185			0.218		
rs17782313	T	0.781	0.817			0.780		
<i>MC4R</i> T>C	C	0.212	0.183	0.799 (0.55-1.14)	0.221	0.220	1.005 (0.75-1.33)	0.972

Abbreviations: SNP ID; single nucleotide polymorphism identification; OR, odds ratio; CI, confidence interval.

*Phenotypic groups was determined using age and sex specific BMI cut-offs provided by the International Obesity Task Force (IOTF).

Logistic regression was used to compare genotype distribution. *p*-values (asymptotic *p*-value for t-statistic) shown are for an additive model and are adjusted for age and sex. OR is shown for the minor allele.

p-value significant ($p < 0.05$) are in bold and italic.