

Association of polymorphisms in 5-HTT (SLC6A4) and MAOA genes with measures of obesity in young adults of Portuguese origin

Helena Dias, Magdalena Muc, Cristina Padez, Licínio Manco

Research Centre for Anthropology and Health (CIAS), Department of Life Sciences,
University of Coimbra, Portugal

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Correspondence:

Licínio Manco, PhD
Research Centre for Anthropology and Health (CIAS)
Department of Life Sciences
University of Coimbra
3000 Coimbra (Portugal)
Phone: +351-239-829051
Fax: +351-239-823491
E-mail: lmanco@antrop.uc.pt

ABSTRACT

Objectives: To investigate the association of polymorphisms in *SLC6A4* and *MAOA* genes with overweight (including obesity).

Material and Methods: Young adults (N=535) of Portuguese origin were genotyped for the *SLC6A4* polymorphisms 5-HTTLPR and STin2 and a *MAOA* VNTR. BMI and body fat percentage were measured, and a questionnaire was used to assess individual's sport practicing habits.

Results: In whole sample, haplotype-based analysis revealed significant association with overweight/obesity for the individual 5-HTTLPR/Stin2 haplotype L10 (P=0.04). In men, the *MAOA* 3R genotype was nominally associated with body fat (P=0.04). In inactive individuals, overweight/obesity was found significantly associated with 5-HTTLPR L-allele (P=0.01) and nominally associated with STin2 10-allele (P=0.03). A significant association was also found testing for all haplotype effects ($\chi^2=8.7$; P=0.03).

Conclusions: We found some evidences for the association of *SLC6A4* and *MAOA* genes with measures of obesity. Our results suggest physical inactivity accentuates the influence of *SLC6A4* polymorphisms on obesity risk.

Introduction

Obesity is a growing worldwide problem, contributing to the increased risk of chronic diseases, such as hypertension, type 2 diabetes mellitus, cardiovascular diseases, cancer and other co-morbidities (Swinburn *et al.*, 2011). Obesity is the result of a combination of factors acting at multiple levels in the current “obesogenic” environment. Epidemiological studies show that genetic factors also play an important role in its development (Xia & Grant, 2013). The recent advent of Genome-Wide Association Studies (GWAS) approach showed more than 75 *loci* associated with at least one obesity-related trait (Lu & Loos, 2013) and new *loci* are still being identified (Locke *et al.* 2015; Shungin *et al.*, 2015).

The potential association between obesity and specific candidate genes related to the central nervous system serotonergic functions has also been explored. Some studies reported that genetic modulation of the serotonergic pathway by polymorphic regions in the serotonin transporter gene (5-HTT; Gene Symbol: *SLC6A4*; chr: 17q11.2) and in the monoamine oxidase A (*MAOA*; chr: Xp11.3) gene may play a role in the development of obesity, although results are still controversial (Ducci *et al.*, 2006; Fuemmeler *et al.*, 2008; Sookoian *et al.*, 2007, 2008; Peralta *et al.*, 2012; Wallmeier *et al.*, 2013). The serotonin transporter 5-HTT is a key molecule in the regulation of serotonin (5-HT) levels via transport of serotonin molecules from the synaptic cleft into presynaptic neurons (Blakely *et al.*, 1994). The MAOA isozyme plays a major role in the metabolism of serotonin in the nerve terminal, which also degrades dopamine and norepinephrine (Shih *et al.*, 1999).

Two polymorphic regions influencing the transcription activity of the *SLC6A4* gene have been mainly studied. The serotonin transporter linked polymorphic region (5-HTTLPR) is located 1 kb upstream the transcription start site, and consists of a number of 20 to 23 base pair (bp) repeat units varying from 13 to 22 units. Within the 5-HTTLPR a 43 bp insertion/deletion (ins/del) gives rise to the most common repeat elements 14R (Short) and 16R (Long) alleles (Heils *et al.*,

1996). The second 5-HTT polymorphic region is a multiallelic 17-bp variable number of tandem repeats sequence (VNTR) in the second intron (named STin2) with two common alleles with 10 and 12 repeats, and rare alleles with 9 and 7 repeats (Lesch *et al.*, 1994; MacKenzie and Quinn, 1999). The promoter region of *MAOA* contains a 30 bp VNTR consisting of 2, 3, 3.5, 4, or 5 repeated copies (Sabol *et al.*, 1998).

In this cross-sectional study, a total of 535 healthy young adults, randomly recruited from central region of Portugal, underwent genotyping for the two polymorphisms in the *SLC6A4* gene (5-HTTLPR and Stin2) and the VNTR *MAOA*, to evaluate associations with overweight and obesity.

Material and Methods

Study sample and measurements:

Our study sample was originally recruited for a study on the influence of physical activity on the association between a FTO variant and adiposity, which have been reported elsewhere (Muc et al., 2015). The present sample included 535 healthy young adults, of European Portuguese descent, mainly from central region of Portugal, being 225 males and 310 females aged between 17-36 years (mean age 20.8 years). Participants were randomly recruited from students at the University of Coimbra between September 2013 and February 2014.

Height (cm) was measured using a stadiometer with the head positioned according to the Frankfort plane and weight was measured via an electronic scale with a precision of 100 g. Anthropometric measures were taken barefoot or in thin socks and in minimal clothing. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Cut-off points defined by WHO were used to define underweight ($<18.5 \text{ kg}/\text{m}^2$), normal weight ($18.5 \leq \text{BMI} < 25 \text{ kg}/\text{m}^2$), overweight ($25 \text{ kg}/\text{m}^2 \leq \text{BMI} < 30 \text{ kg}/\text{m}^2$) and obesity ($\text{BMI} \geq 30 \text{ kg}/\text{m}^2$). Body composition (percentage of fat) was measured using the bioelectric impedance method (Bodystat 1500MDD).

Physical activity was assessed using the International Physical Activity Questionnaires (IPAQ) (Pardini et al., 2001) and participation in regular physical activity was assessed through the question, "Do you currently practice any sport?" A dichotomous variable was created with answers of "yes" or "no".

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and with the institutional and ethical guidelines of the University of Coimbra. Written informed consent was obtained from all participating subjects.

Genotyping:

Buccal swabs of volunteer individuals were submitted to DNA extraction, using the FavorPrep™ Genomic DNA Mini Kit (Favorgen® Biotech Corp, Taiwan). Genotyping was performed by Polymerase Chain Reaction (PCR) followed by electrophoresis in 2.5% agarose gels with ethidium bromide. Primers for the 5-HTTLPR polymorphism were described in Gelernter et al. (1997), (JP 5'-ATGCCAGCACCTAACCCCTAATGT-3' and GR 5'-GGACCGCAAGGTGGGCGGGA-3'). For STin2 the PCR amplification of the 17-bp VNTR region located in intron 2 of *SLC6A4* was performed from genomic DNA using forward 5'-GGTCAGTATCACAGGCTGCGAGTAG-3' and reverse 5'-TGTTCTAGTCTTACGCCAGTGAAG-3' primers described in Uzun et al. (2015). The VNTR in *MAOA* gene was studied using the primers 5'-ACAGCCTGACCGTGGAGAAG-3' (forward) and 5' - GAACGTTGACGCTCCCGGA - 3' (reverse), as described in Sabol *et al.* (1998).

Statistical Analysis:

Genotype and allele frequencies, Hardy-Weinberg equilibrium probability values, as well as the linkage phase from diploid data by statistical inference via ELB algorithm for 5-HTTLPR and Stin2 polymorphisms were obtained using the software Arlequin, ver 3.01 (Excoffier et al., 2005; <http://cmpg.unibe.ch/software/arlequin3/>).

The association between genotype distributions and overweight/obesity was tested by logistic regression under an additive genetic model, adjusted for sex and age, and presented as odds ratios (OR) with 95% confidence intervals (CI) and P-values. The control group (BMI <25 kg/m²) was composed with 425 subjects (260 females and 165 males) and the 110 subjects defined as overweight or obese (BMI ≥25 kg/m²) (50 females and 60 males) were merged in the case group. Genotypes with the 2R, 3.5R and the 5R variants of the *MAOA* polymorphism and the 9 repeats allele of the Stin2 polymorphism were not included in the association analysis due to their low frequency in the population. The haplotype associations combining 5-HTTLPR and Stin2 polymorphisms were assessed by chi-square analysis. Linear regression models were used to test the association of the studied polymorphisms with obesity-related quantitative traits BMI

and FAT %. A significant P-value was considered below 0.017 (0.05/3) by applying a Bonferroni correction for multiple testing, and a P-value between 0.017 and 0.05 has been considered nominally significant. The interaction of physical activity with polymorphisms for BMI and FAT % quantitative traits was detected by comparing the difference between two regression coefficients. All these statistical analyses were done by using the set-based tests implemented on PLINK software v.1.07 (Purcell et al., 2007; <http://pngu.mgh.harvard.edu/purcell/plink/>).

Results

Population characteristics

As summarised in Table 1, participants were 57.9% women and 42.1% males. The study sample included 23 underweight (mean BMI 17.6 kg/m²), 402 normal-weight (mean BMI 21.8 kg/m²), 88 overweight (mean BMI 27 kg/m²) and 22 obese subjects (mean BMI 33.8 kg/m²), allowing a prevalence of 16.5% for overweight and 4.1% for obesity. Subjects practicing sports reported a mean of 303 minutes of vigorous physical activity per week (that includes sport practice or heavy physical work) and subjects without practicing sport report a mean of 80 minutes.

Population genetics

The genotyping success rate was 99.1% for 5-HTTLPR, 99.8% for Stin2 and 93.5% for the *MAOA* VNTR polymorphism. Genotypes and allele frequencies for the three studied polymorphisms and haplotypes for the 5-HTTLPR and Stin2 combinations are detailed in Table 2. The genotype distributions were in Hardy-Weinberg equilibrium for polymorphisms 5-HTTLPR and Stin2 ($P=0.308$ and $P=0.243$, respectively) in the whole population as also for the *MAOA* VNTR in women ($P=0.869$).

Associations with measures of obesity

For the association studies we merged overweight and obese subjects in one group and the logistic regression, in the additive model, revealed in the overall population no statistically significant associations with overweight/obesity for both the *SLC6A4* polymorphisms 5-HTTLPR and Stin2 or for *MAOA* VNTR polymorphism ($P>0.05$ in non-adjusted and adjusted models) (Table 3). However, a nominal non-adjusted near significant association ($P=0.07$) was found between the Stin2 10 allele and overweight/obesity. The haplotype analysis combining the two

SLC6A4 5-HTTLPR and *Stin2* polymorphisms, located at a distance of about 16 Kb and in low linkage disequilibrium (LD) ($r^2=0.07$), revealed no significant association testing for all haplotype effects ($\chi^2=0.014$; $p=0.90$); however, the individual haplotype L10 showed a statistical significant association ($\chi^2=4.2$; $p=0.04$) with a frequency of 0.339 in the overweight/obese group vs. 0.268 in controls (Table 4).

Considering that in a previous work Muc et al. (2015) have shown that physical activity attenuate the genetic susceptibility to obesity in this same cohort of individuals, we split the population sample by sport practice. In inactive individuals the logistic regression showed a non-adjusted significant association with overweight/obesity for 5-HTTLPR L-allele ($P=0.01$) and a nominal association for the *STin2* 10-allele ($P=0.03$) (Table 3). A nominal near significant P-value ($P=0.05$) was also observed for 5-HTTLPR adjusted for age and sex (Table 3). Moreover, a significant association ($\chi^2=8.7$; $P=0.03$) was found testing for all haplotype effects, with haplotype L10 associated with risk of overweight/obesity ($\chi^2=5.1$; $P=0.02$) (frequency of 0.367 in the overweight/obese group vs. 0.273 in controls) and haplotype S12 as a protective haplotype ($\chi^2=7.4$; $P=0.006$) (frequency of 0.366 in controls vs. 0.248 in the overweight/obese group) (Table 4). In contrast, in group reporting practicing sports no significant associations were found for the two *SLC6A4* 5-HTTLPR and *Stin2* polymorphisms ($P>0.05$) (Table 3). For the *MAOA* VNTR polymorphism, no significant associations were found in both groups split by sport practicing (Table 3).

BMI and fat percentage quantitative traits were also tested for associations with each of the studied polymorphisms 5-HTTLPR, *Stin2* and *MAOA* VNTR, in a linear regression framework. No significant effects were observed for BMI although a nominal near significant association was found for *Stin2* ($P=0.08$). Regarding fat percentage, a nominal significant association ($P=0.03$) was observed in men for the *MAOA* VNTR even adjusted for age and sex ($P=0.04$).

Testing for GxE effects we detected a significant interaction between the *Stin2* polymorphism and physical activity that affects BMI ($P_{\text{interaction}}=0.03$) (Table 5). In concordance, when assessing the relationship between BMI and polymorphisms stratifying according physical activity, among subjects reporting not-practicing sport the 5-HTTLPR L-allele and *STin2* 10-allele were found nominally, respectively, borderline associated ($P=0.07$) and associated ($P=0.03$) with greater BMI. In contrast, BMI was not significantly associated with the two *SLC6A4* polymorphisms in subjects reporting sport practicing ($P>0.05$).

Discussion

In our study, two polymorphic regions in the *SLC6A4* gene (5-HTTLPR and Stin2 VNTR polymorphisms) and the upstream VNTR polymorphism in the *MAOA* gene, both genes involved in the serotonergic pathway, were analysed for associations with measures of overweight/obesity. Obtained allele frequencies for the studied polymorphisms were found similar to those in previous reports for European populations (Haberstick et al., 2015; Murdoch et al., 2013; Noskova et al., 2008; Ogilvie et al., 1996).

In the overall population our findings report no significant associations with overweight/obesity for the 5-HTTLPR and Stin2 polymorphism, although several nominal near significant associations were found with overweight/obesity or BMI and body fat percentage. Moreover, a significant association with the risk of overweight/obesity was observed for the individual haplotype 5-HTTLPR/Stin2 L10 ($P=0.04$).

When the study population was split by sport practicing, significant associations were observed among inactive individuals between both *SLC6A4* polymorphisms and overweight/obesity but not in a group reporting participation in sports. In group of less active individuals, each copy of the risk allele increased the odds of being overweight/obese by 58% for 5-HTTLPR ($P=0.01$) and 47% for Stin2 ($P=0.03$) in a non-adjusted model. Moreover, a formal test for genotype x physical activity interaction achieved statistical significance for Stin2 on BMI (interaction $P=0.03$). This pattern of results indicates physical inactivity may accentuates the influence of *SLC6A4* variants in obesity risk, providing evidences for the interplay between genes and lifestyle as was also previously shown in this cohort for the *FTO* gene (Muc et al., 2015).

The observed significant associations with the risk of obesity when testing for all haplotype effects or individual haplotypes, both in overall and among inactive individuals, is in concordance with previous studies showing haplotype-based analysis can be much more powerful than marker-by-marker analysis in association studies (Zhang et al. 2002).

The obtained results for the *SLC6A4* gene polymorphisms are in agreement with the previous study of Bah *et al.* (2010) in a Swedish population showing that the 5-HTTLPR SS genotype is more frequent in underweight subjects. Also Peralta *et al.* (2012), in a Mexican population sample, reported the L allele associated with overweight/obesity. As the 5-HTTLPR S allele is associated with a lower transcriptional activity resulting in reduced reuptake of serotonin in synaptic cleft (Heils *et al.*, 1996), it was argued that this allele is associated with higher availability of 5-HT in central serotonergic synapses and this should intensify satiety, reducing food intake and inducing lower BMI and fat mass (Peralta *et al.* 2012). However, these results differ from those studies reporting the S allele associated with higher BMI (Fuemmeler *et al.*, 2008, Sookoian *et al.*, 2008; 2007), or reporting no associations between 5-HTTLPR and obesity traits (Mergen *et al.*, 2007; Hamed *et al.*, 2015). Regarding the VNTR STin2 in the second intron of *SLC6A4* gene, only one recent study has investigated the association with obesity and no significant results were observed (Uzun *et al.*, 2015).

The *MAOA* VNTR polymorphism analysis revealed no significant associations with overweight/obesity neither in the overall population nor in both sub-groups split per sport practicing. However, in men the 3R genotype was found nominally associated with higher fat percentage ($P=0.03$). The common VNTR polymorphism in the promoter region of *MAOA* gene has been previously examined for associations with obesity in at least four studies and our results are in agreement with these previous reports. In white UK women, significant associations were detected for BMI, with the obese group showing an excess of the low activity genotype (3R-3R) when compared to the non-obese group (Need *et al.*, 2006). Also Ducci *et al.* (2006) found a significant association between variation in BMI and *MAOA* genotypes, with the low-activity allele associated with a higher BMI among a sample of primarily non-obese men with and without a history of alcohol dependence. Wallmeir *et al.* (2013) showed, in a longitudinal study with non-related Caucasian subjects with severe obesity, that body weight

did not vary between the different *MAOA* VNTR genotypes for either men or women, but female carriers of the 3R genotype were found to have a significantly higher BMI at the end of the program. Finally, in a study by Fuemeller et al. (2008) no associations with overweight/obesity were found in women, however, among men, the low activity *MAOA* VNTR allele was found to be associated with overweight/obesity.

Until now, there is no consensus in the literature about the functional relationships between the *SLC6A4* or *MAOA* genes and obesity/overweight. Both 5-HTTLPR and *MAOA* polymorphisms have also been associated with depression in previous studies (Caspi et al., 2003; Bonvicini et al., 2010), and it has been shown that depression is a likely contributor to obesity, particularly in women (Scott et al. 2008; Anderson et al. 2007). Thus, modulation of the serotonergic pathway by either 5-HTTLPR or *MAOA* polymorphisms could lead to elevated depression symptoms and ultimately to a reduced weight loss and/or maintenance (Wallmeier et al., 2013).

Our study, despite some significant findings, has several limitations. The overall sample size is low and it is known that a study with low statistical power (because of low sample size, small effects or both) has a reduced chance of detecting statistically significant results and negatively affects the likelihood that a nominally statistically significant finding reflects a true effect (Button et al., 2013). Furthermore, it is described for the 5-HTLLPR polymorphism that the L allele in association with the near upstream SNP rs25531 G allele lead to a lower transcriptional activity similar to that of the S allele (Hu et al., 2006) and this SNP was not assessed in this work.

Conclusions: We found several evidences for the association between *SLC6A4* and *MAOA* genes and measures of overweight/obesity. Moreover, we show physical inactivity accentuates the influence of *SLC6A4* variants on obesity risk. Future studies are required to clarify the role of

SLC6A4 and *MAOA* genes in obesity risk. Thus, results still need to be replicated among different populations to improve knowledge and clarification.

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Declaration of Interest statement:

The authors report no declarations of interest.

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Table 1: Characteristics of the study sample and the distribution comparison between sexes.

Parameters	Overall	Women	Men
N (%)	535	310 (57.9)	225 (42.1)
Mean Age (years)	20.68 (2.65)	20.56 (2.49)	21.09 (2.84)
Anthropometric			
Weight (kg)	64.32 (12.64)	58.44 (9.37)	72.04 (12.23)
Height (m)	1.67 (0.09)	1.61 (0.06)	1.74 (0.07)
BMI (kg/m ²)	23.00 (3.60)	22.51 (3.42)	23.67 (3.66)
Body fat (%)	22.81 (7.50)	27.73 (5.18)	16.36 (4.64)
Underweight (<18.5 kg/m ²)*	23 (4.3)	18 (5.8)	5 (2.2)
Normal weight (≥18.5 to <25 kg/m ²)*	402 (75.1)	242 (78.1)	160 (71.1)
Overweight (≥25 kg/m ² to <30 kg/m ²)*	88 (16.5)	38 (12.3)	50 (22.2)
Obese (≥30 kg/m ²)*	22 (4.1)	12 (3.9)	10 (4.4)
Physical activity			
Practicing sport *	146 (27.3)	57 (18.4)	89 (39.6)
Not-Practicing sport *	389 (72.7)	253 (81.6)	136 (60.4)

Abbreviations: BMI, body mass index; N, number of individuals.

Data presented as Mean (SD) for continuous anthropometric variables and as N (%) for categorical variables (*).

Table 2: Genotype, allele and haplotype frequencies for the two 5-HTT polymorphisms 5-HTTLPR and STin2 and for the MAOA gene VNTR polymorphism in the sample of Portuguese young adults.

Polymorphisms	Genotypes N (freq)	Alleles N (freq)	P HWE
5-HTTLPR (N=530)	LL: 170 (0.321) LS: 249 (0.470) SS: 111 (0.209)	L: 0.556 S: 0.444	0.308
STin2 (N=523)	12-12: 197 (0.376) 12-10: 233 (0.446) 10-10: 87 (0.166) 12-09: 5 (0.010) 10-09: 1 (0.002)	12: 0.604 10: 0.390 09: 0.006	0.243
Haplotypes 5-HTTLPR/STin2 (N=518)	L 12: 252 (0.243) S 12: 371 (0.358) L 10: 319 (0.308) S 10: 88 (0.085) L 09: 6 (0.006)		
VNTR MAOA Women (n=291)	4/4: 126 (0.433) 4/3: 124 (0.426) 3/3: 35 (0.12) 5/4: 3 (0.01) 5/3: 1 (0.003) 4/2: 1 (0.003) 3/2: 1 (0.003)	4: 0.653 3: 0.337 5: 0.007 2: 0.003	0.869
VNTR MAOA Men (n=209)	4: 136 3: 68 5: 3 2: 1 3.5: 3	4: 0.651 3: 0.325 5: 0.014 2: 0.005 3.5: 0.005	-

Abbreviations: N, number of genotyped samples; Freq, frequency; HWE, Hardy-Weinberg Equilibrium.

Table 3: Association of VNTR polymorphisms in 5-HTT (5-HTTLPR and STin2) and MAOA genes with the risk of overweight/obesity in the sample of Portuguese young adults.

Groups	Locus (N)	Alleles	Normal N (freq)	OB/OW N (freq)	OR (95% CI)	P ^a	P ^b
Overall	5-HTTLPR (530)	S	384 (0.46)	87 (0.40)	0.78 (0.58-1.05)	0.11	0.32
		L	456 (0.54)	133 (0.60)			
	STin2 (517)	10	309 (0.38)	98 (0.45)	1.32 (0.98-1.77)	0.07	0.14
		12	507 (0.62)	120 (0.55)			
MAOA F (285)	3	168 (0.35)	26 (0.30)	0.819 (0.50-1.34)	0.42	0.35	
	4	316 (0.65)	60 (0.69)				
MAOA M (204)	3	47 (0.31)	21 (0.39)	0.689 (0.36-1.02)	0.26	0.28	
	4	104 (0.69)	32 (0.60)				
Practicing sport	5-HTTLPR (146)	S	92 (0.41)	32 (0.48)	1.43 (0.79-2.06)	0.23	0.27
		L	134 (0.59)	34 (0.52)			
	STin2 (139)	10	82 (0.39)	26 (0.39)	1.03 (0.60-1.77)	0.92	0.90
		12	130 (0.61)	40 (0.61)			
MAOA F (52)	3	36 (0.46)	8 (0.31)	0.47 (0.17-1.32)	0.15	0.20	
	4	42 (0.54)	18 (0.69)				
MAOA M (82)	3	44 (0.34)	12 (0.35)	1.03 (0.59-1.81)	0.91	0.96	
	4	86 (0.66)	22 (0.65)				
Not-practicing sport	5-HTTLPR (384)	S	292 (0.48)	55 (0.36)	0.64 (0.45-0.91)	<u>0.01</u>	0.05
		L	322 (0.52)	99 (0.64)			
	STin2 (378)	10	227 (0.38)	72 (0.47)	1.47 (1.03-2.09)	<u>0.03</u>	0.15
		12	377 (0.62)	80 (0.53)			
MAOA F (233)	3	132 (0.33)	18 (0.3)	0.895 (0.50-1.5)	0.71	0.61	
	4	274 (0.67)	42 (0.7)				
MAOA M (122)	3	25 (0.29)	15 (0.42)	0.574 (0.16-0.99)	0.18	0.14	
	4	61 (0.71)	21 (0.58)				

Abbreviations: OB/OW, Obese/Overweight; Freq, frequency; OR, odds ratio; CI, confidence interval; N, number of samples / alleles; F, females; M, males; na, not applicable.

The OR and P-values were obtained by logistic regression under an additive model considering the minor allele as the reference.

The OR was estimated for the minor allele and P-values were unadjusted (P^a) and adjusted for age and sex (P^b).

The nominal significant results (P<0.05) are in bold and underlined.

Table 4: Haplotype frequencies for the two 5-HTT VNTR polymorphisms 5-HTTLPR and STin2 in the overall population and not-practicing sport group and their association with the risk of overweight/obesity.

Groups	Haplotype	Normal group	OB/OW group	χ^2	P ^a	χ^2	P ^b
Overall	S10	0.114	0.111	0.01	0.904	0.014	0.90
	L10	0.268	0.339	4.2	<u>0.041</u>		
	S12	0.344	0.284	2.8	0.093		
	L12	0.274	0.267	0.04	0.837		
Practicing sport	S10	0.134	0.129	0.01	0.91	2.062	0.56
	L10	0.252	0.264	0.04	0.84		
	S12	0.281	0.355	1.34	0.24		
	L12	0.333	0.251	0.33	0.21		
Not practicing sport	S10	0.107	0.107	0.0002	0.988	8.786	<u>0.03</u>
	L10	0.273	0.367	5.1	<u>0.024</u>		
	S12	0.366	0.248	7.4	<u>0.006</u>		
	L12	0.254	0.278	0.4	0.544		

Abbreviations: OB/OW, Obese/Overweight; χ^2 , Chi-square.

Data for haplotypes with frequencies > 0.01.

P-values were for each haplotype versus all others (P^a) and testing all haplotype effects (P^b).

Nominal significant results (P<0.05) are in bold and underlined.

Table 5: Association of 5-HTT and MAOA polymorphism with quantitative traits BMI and FAT% in the studied population based on linear regression models and the interaction P values between polymorphisms and physical activity based on sport practicing.

<i>Locus</i>	<i>Trait</i>	<i>N</i>	β	SE	P^a	P^b	P_{int}
5-HTTLPR	BMI	530	-0.353	0.215	0.10	0.39	0.29
	FAT%	513	0.905	0.461	0.05	0.48	0.12
STin2	BMI	517	0.385	0.221	0.08	0.18	<u>0.03</u>
	FAT%	500	-0.724	0.473	0.12	0.49	0.75
VNTR MAOA Women	BMI	285	-0.242	0.242	0.39	0.35	0.35
	FAT%	270	-0.180	0.455	0.69	0.66	0.16
VNTR MAOA Men	BMI	204	0.399	0.274	0.15	0.16	0.16
	FAT%	203	0.724	0.342	<u>0.03</u>	<u>0.04</u>	0.79

Abbreviations: BMI, body mass index; FAT %, body fat percentage; N, number of analysed samples.

Table includes the effect sizes (regression coefficient beta, $\hat{\alpha}$) of the minor allele, standard error (SE) and P-values (asymptotic P-value) for quantitative traits, unadjusted (P^a) and adjusted for age and sex (P^b), as well as the interaction P values (P_{int}) between polymorphisms and physical activity.

Nominal significant results ($P < 0.05$) are in bold and underlined.