Influence of Physical Activity on the association between the FTO variant rs9939609 and Adiposity in young adults

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ABSTRACT

Objectives: To investigate in a population sample of Portuguese young adults the association of the *FTO* variant rs9939609 with obesity, BMI and body-fat and interaction with physical activity (PA) on obesity-susceptibility.

Methods: SNP rs9939609 A/T was genotyped in 550 subjects (231 males and 319 females; 18–36 year-old; mean age 21 year-old) by TaqMan assay. PA was assessed with a validated self-reported questionnaire of IPAQ.

Results: We replicated the association of rs9939609-A risk allele with BMI (*P*=0.04) and fat-mass (*P*=0.031), and with overweight (including obesity) under a recessive model (*P*=0.034). Stratified analyses showed i) a significant association with overweight/obesity in inactive individuals (*P*=0.02) but not in a group reporting sport practicing (*P*=0.97). Spearman's correlation test suggested that the impact of a successive increase in PA is a decrease in the body-fat percentage (r=-0.16; *P*=0.0002), which is more accentuated for homozygous AA (r=-0.34; *P*=0.002), and an increase in BMI (r=0.14; *P*=0.001), with a statistically significant correlation for homozygous TT (r=0.22; *P*=0.002).

Conclusions: This study reveals interactions between rs9939609 and PA on obesity indices in Portuguese young adults, suggesting a change in the different body components (lean and fat mass) depending on the FTO genotypes.

Keywords: *FTO* variant rs9939609; BMI and body-fat; obesity and physical activity; Portuguese young adults.

INTRODUCTION

The first common genetic variants undoubtedly related with susceptibility to obesity were identified in 2007 among individuals of European ancestry in the first intron of the human *FTO* (fat mass and obesity associated) gene (chromosome 16q12.2) (Frayling et al., 2007; Dina et al., 2007; Scuteri et al., 2007). From all the *FTO* single nucleotide polymorphisms (SNPs) located within the obesity-associated region spanning introns 1 and 2, the most widely replicated is rs9939609.

The *FTO* gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase (Gerken et al., 2007) but is also a transcriptional coactivator (Wu et al., 2010) and a possible regulator of telomere length (Dlouha et al., 2012). *FTO* is abundantly expressed in the hypothalamus, and epidemiological and functional studies suggested to be directly involved in the regulation of energy intake (Cecil et al., 2008; Haupt et al., 2009). Moreover, *FTO* is expressed in multiple tissues such as white adipose tissue, skeletal muscle and liver, also having direct influences on adipose tissue metabolism (Grunnet et al., 2009).

Several recent studies suggested that physical activity may attenuate the influence of genetic factors on development of obesity. The most extensively studied example of a gene interaction with physical activity in obesity is for the *FTO* locus, concluding in general that physical inactivity accentuates the association of *FTO* variants with obesity-related traits (Andreasen et al., 2008; Rampersaud et al., 2008; Lee et al., 2010; Ruiz et al., 2010; Xi et al., 2011). However, this gene *versus* physical activity interaction in obesity was not yet assessed in a young adult population.

In the Portuguese population, the prevalence of overweight and obesity has increased during the last decade reaching values of 40% in children of 7-9 years-old (Cardoso and Padez, 2000; Padez et al., 2004). Recent genetic association studies in

children (Albuquerque et al., 2013) and adult females (Carlos et al., 2013) replicated the association of FTO variants with obesity and obesity-related traits, however no interaction with physical activity was assessed. In this study, we examined in a population sample of Portuguese young adults, including males and females, i) the association of *FTO* variant rs9939609 with obesity, BMI and body fat mass, and ii) the interaction between rs9939609 and physical activity on obesity-susceptibility.

MATERIAL AND METHODS

Study sample and measurements: The present study included 550 healthy young adults, 231 males and 319 females (aged 18-36 year-old; mean age 21 year-old) of European Portuguese descent, mainly from central region of Portugal, randomly recruited from students at the University of Coimbra between September 2013 and February 2014. Cut-off point defined by WHO were used to define normal weight (BMI <25 kg/m^2), overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2) and obesity (BMI ≥30 kg/m^2). Body composition (percentage of fat) was measured using the bioelectric impedance method. 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". The MET (Metabolic Equivalent of Task) minutes were calculated for the total physical activity per week calculated according to the International Physical Activity Questionnaires (IPAQ).

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: A buccal swab sample was collected from each subject and genomic DNA was extracted by using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen® Biotech Corp, Tawain). The FTO rs9939609 polymorphism was genotyped by TaqMan assay.

Statistical analysis: Allelic and genotypic frequencies were estimated by direct counting. Hardy-Weinberg equilibrium p-value was achieved using an exact test. Linear regression models were used to test the association of the rs9939609 polymorphism with obesity-related quantitative traits. Logistic regression, under an additive genetic model, as also a recessive genetic model, were used to test the risk of being overweight (including obese), repeated split by "practicing" or "not-practicing" sport. These statistical analyses were done using the set-based tests implemented on PLINK software v.1.07 (http://pngu.mgh.harvard.edu/purcell/plink/).

We further determined the correlation between BMI and percentage of fat with physical activity, and whether the association of weekly physical activity measured as the total MET minutes on BMI and fat percentage was modified by genotypes, using Spearman's correlation test. Rank variables of the continuous data have been created in order to illustrate non-parametric Spearman correlations. These statistical analyses were performed using the SPSS software, version. 21.0.

P-values <0.05 were considered as statistical significant.

(Detailed description of the measurements, genotyping and statistical analyses can be found in Supporting Information – S-Material and Methods).

RESULTS

Characteristics of the study sample distributed by sex are shown in Supporting Information S-Table 1. The study sample included 434 normal-weight, 92 overweight

and 24 obese subjects allowing a prevalence of overweight and obesity of 21.1% (4.4% classified as obese). A total of 27.8% of the participants practiced some sport (18.5% of all women and 40.5% of all men).

The allele frequencies observed for *FTO* rs9939609 polymorphism in the total sample were 0.385 for A-allele (0.615 for T-allele), in accordance with those found in the HapMap CEU population (http://www.ensembl.org/). Genotype distributions were in agreement with Hardy-Weinberg equilibrium (P=0.653).

Association between the *FTO* rs9939609 polymorphism and obesity-related parameters

Table 1 shows the association results between *FTO* rs9939609 genotypes and obesity-related quantitative traits, in a linear regression framework. Statistical significant results were observed for BMI (P=0.040) and body fat percentage (P=0.031), after adjustment for age and sex, with rs9939609 per-A allele effects of 0.46 kg/m^2 on BMI and 0.67 % on body fat.

Logistic regression analysis, in the additive model, revealed for the total population that the *FTO* rs9939609 minor A-allele was near associated with the overweight (including obesity), adjusting for sex and age (OR=1.32; P=0.064) (Table 2). Moreover, the recessive genetic model showed significant association between the rs9939609 polymorphism and the overweight/obese phenotype (X^2=4.47; P=0.034). Accordingly, 21.6% of the overweight/obese individuals were homozygous AA compared to 13.6% of the lean subjects.

Interaction between the *FTO* rs9939609 polymorphism and physical activity on adiposity

The logistic regression analysis, under the additive model, testing overweight/obesity associations for the population split per sport practicing (Table 2) revealed for the group not-practicing sport a significantly increased risk for the minor A-allele (OR=1.54, *P*=0.02). A similar result was observed when considering the recessive model of genetic association (X^2=7.11; *P*=0.007). In this stratum, 25.3% of the overweight/obese individuals were homozygous AA compared to 13.2% of normal weight status subjects. In contrast, no such association was observed in group reporting sport activity: *P*=0.97 for logistic regression and *P*=0.84 for the recessive model.

Figure 1 graphically illustrates, using predicted values from the Spearman test, the correlation between both BMI and fat percentage with MET-minutes of PA. Significant correlations, in the absence of genetic effects, were observed for the total sample: Spearman's correlation test suggested that the impact of a successive increase in PA is an increase in BMI (r=0.14; *P*=0.001) (Figure 1A) and a decrease in body-fat percentage (r=-0.16; *P*=0.0002) (Figure 1B). Stratifying by the rs9939609 genotypes, a larger difference across FTO genotypes was observed for BMI and fat percentage in less physically active subjects, with the AA homozygous subjects showing the large values. Moreover, Figure 1C reveal a positive correlation between BMI and PA level with the most accentuated and significant correlation for the TT homozygous genotype (r=0.218; *P*=0.002) (homozygous AA r=0.681, *P*=0.046; heterozygous TA r=0.108, *P*=0.087). On the other hand, a negative correlation between PA and fat percentage (Figure 1D) was observed, statistical significant for homozygous AA (r=-0.339; *P*=0.002) and heterozygous TA (r=-0.161; *P*=0.011) genotypes but not for homozygous TT (r=-0.079; *P*=0.274).

DISCUSSION

In this study, we investigated in a sample of Portuguese young adults (mean age of 21 year-old) the association of *FTO* variant rs9939609 with obesity, BMI and body-fat as well as the interaction between rs9939609 and physical activity on obesity-susceptibility.

The young adult study sample revealed a prevalence of overweight (16.7%) and obesity (4.4%) similar to that previously reported for Portuguese adults within a similar age span: men 26.9% overweight and 4.4% obesity; women: 15.6% overweight and 2.4% obesity (Carreira et al., 2012).

Significant associations were observed for BMI (*P*=0.04) and body fat percentage (*P*=0.031), as also for the overweight (including obesity) phenotype under a recessive genetic model (*P*=0.034). Carriers of the FTO rs9939609 A-allele reflect a BMI increased by 0.46 kg/m^2 (p=0.04) for the whole population consistent with previous studies showing that each risk allele increases BMI by ~0.40–0.66 kg/m^2 (Loos and Bouchard, 2008). While BMI is generally a good indicator of overall adiposity, it does not distinguish fat from lean mass. Thus, we further analyzed the body-fat percentage as a more accurate estimate of body adiposity, and indeed an increase of 0.67 % (*P*=0.031) in the fat percentage, per A-allele of rs9939609 was observed.

In this study sample of young adults we found an interaction between the *FTO* rs9939609 variant and self-reported sport activity on the risk of overweight/obesity whereas a significant association (*P*=0.02) was found in subjects without practicing sport but not in those reporting practicing sport activity (*P*=0.97). Thus, our data suggests an FTO genotype's association with obesity being more pronounced in inactive than in active subjects. This observed moderation effect of physical activity on the genetic susceptibility to obesity is in accordance with previous data of gene *versus* physical activity interactions showing that low physical activity seems to accentuate the

effect of FTO risk alleles on body fat accumulation both in adults (Andreasen et al., 2008; Rampersaud et al., 2008) and children (Ruiz et al., 2010; Xi et al., 2011).

We further observed by using a correlation test that the impact of a successive increase in MET-minutes of physical activity was a decrease in the body-fat percentage, which is more accentuated for subjects with AA genotype, and an increase in the rank of BMI, with a significant correlation for the homozygous TT subjects. This apparently contradictory result may be explained by natural physiological differences, as our study sample includes only young adults (mean age 21 year-old). Contrary to older people and children, young adults have a larger variation in muscle mass and generally higher muscle mass content (Sayer et al., 2008) so the change caused by exercise has an effect on body composition rather than total mass (Westerterp, 2013). As BMI variations are mostly the effect of the muscle to fat mass proportions, the observed significant increase in BMI under physically activity, mainly in young adults homozygous for the major genotype TT, could be due to an increase in lean (muscle) mass rather than in fat mass. Since the homozygous AA individuals have a tendency of fat accumulation under the conditions of low physical activity, which we have also found, their BMI does not change with the increased activity, but we can rather observe the transition from fat to muscle mass. This could explain why we can see in homozygous AA individuals a significant decrease in fat mass associated with higher activity levels but no significant change in total body mass. Individuals with TT genotype, having lower tendency of fat accumulation even when inactive, increase their body mass by building muscle, so the correlation is pronounced for the BMI and not for fat mass.

In summary, our study replicated the association of FTO rs9939609 variant with increased body mass in a population sample of Portuguese young adults and revealed the influence of physical activity on the attenuation of genetic susceptibility to obesity

with the consideration for both lean and fat mass variations. In more physically active groups, we have found no significant association of the FTO variant with overweight/obesity. Moreover, our data suggest a change in the different body components (lean and fat mass) in young adults associated with different levels of physical activity depending on the FTO genotypes. To our knowledge, this is the first study in young adults to report an interaction between the FTO rs9939609 polymorphism and physical activity level on obesity indices.

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LITERATURE CITED

Albuquerque D, Nóbrega C, Manco L. 2013. Association of FTO polymorphisms with obesity and obesity-related outcomes in Portuguese children. PLoS One 8:e54370.

Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, Clausen JO, Sandbaek A, Lauritzen T, Hansen L, Jørgensen T, Pedersen O, Hansen T. 2008. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes 57:95-101.

Cardoso HF, Padez C. 2008. Changes in height, weight, BMI and in the prevalence of obesity among 9 to 11 year-old affluent Portuguese schoolboys, between 1960 and 2000. Ann Hum Biol 35:624-638.

Carlos FF, Silva-Nunes J, Flores O, Brito M, Doria G, Veiga L, Baptista PV. 2013. Association of FTO and PPARG polymorphisms with obesity in Portuguese women. Diabetes Metab Syndr Obes 6:241-245.

Carreira H, Pereira M, Azevedo A, Lunet N. 2012. Effect of the type of population on estimates of mean body mass index and prevalence of overweight and obesity: a systematic review of studies of Portuguese adults. Ann Hum Biol 39:223–238.

Cecil JE, Tavendale R,Watt P, Hetherington MM, Palmer CN. 2008. An obesity-associated FTO gene variant and increased energy intake in children. N Engl J Med 359:2558–2566.

Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 39:724-726.

Dlouha D, Pitha J, Lanska V, Hubacek JA.Association between FTO 1st intron tagging variant and telomere length in middle aged females. 3PMFs study. 2012. Clin Chim Acta 413:1222-1225.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316: 889-894.

Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. 2007. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318:1469–1472.

Grunnet LG, Nilsson E, Ling C, Hansen T, Pedersen O, Groop L, Vaag A, Poulsen P. 2009. Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue. Diabetes 58:2402–2408.

Haupt A, Thamer C, Staiger H, Tschritter O, Kirchhoff K, Machicao F, Häring HU, Stefan N, Fritsche A. 2009. Variation in the FTO gene influences food intake but not energy expenditure. Exp Clin Endocrinol Diabetes 117:194–197.

Loos RJ, Bouchard C. 2008. FTO: the first gene contributing to common forms of human obesity. Obes Rev 9:246–250.

Padez C, Fernandes T, Mourão I, Moreira P, Rosado V. 2004. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: Trends in body mass index from 1970-2002. Am J Hum Biol 16:670-678.

Rampersaud E, Mitchell BD, Pollin TI, Fu M, Shen H, O'Connell JR, Ducharme JL, Hines S, Sack P, Naglieri R, Shuldiner AR, Snitker S. 2008. Physical activity and the association of common FTO gene variants with body mass index and obesity. Arch Intern Med 168:1791–1797.

Ruiz JR, Labayen I, Ortega FB, Legry V, Moreno LA, Dallongeville J, Martínez-Gómez D, Bokor S, Manios Y, Ciarapica D, Gottrand F, De Henauw S, Molnár D, Sjöström M, Meirhaeghe A; HELENA Study Group. 2010. Attenuation of the effect of the FTO rs9939609 polymorphism on total and central body fat by physical activity in adolescents: the HELENA study. Arch Pediatr Adolesc Med 164:328-333.

Sayer AA, Syddall H, Martin H, Patel H, Baylis D, Cooper C. 2008. The developmental origins of sarcopenia. J Nutr Health Aging 12:427–432.

Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrú M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR. 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet 3:1200-1210.

Xi B, Wang C, Wu L, Zhang M, Shen Y, Zhao X, Wang X, Mi J. 2011. Influence of physical inactivity on associations between single nucleotide polymorphisms and genetic predisposition to childhood obesity. Am J Epidemiol 173:1256-1262.

Westerterp KR. 2013. Energy Balance in Motion. Berlin, Heidelberg: Springer Berlin Heidelberg.

Wu Q, Saunders RA, Szkudlarek-Mikho M, Serna Ide L, Chin KV. 2010.The obesity-associated Fto gene is a transcriptional coactivator. Biochem Biophys Res Commun 401:390-395.

Legends

Figure 1:

Correlations between the MET-minutes of the total physical activity and BMI and body fat percentage for the total population sample in the absence of genetic effects (A and C) and per FTO genotype (B and D). To illustrate the non-parametrical Spearman correlation, rank values of all three variables were plotted instead of the actual values.

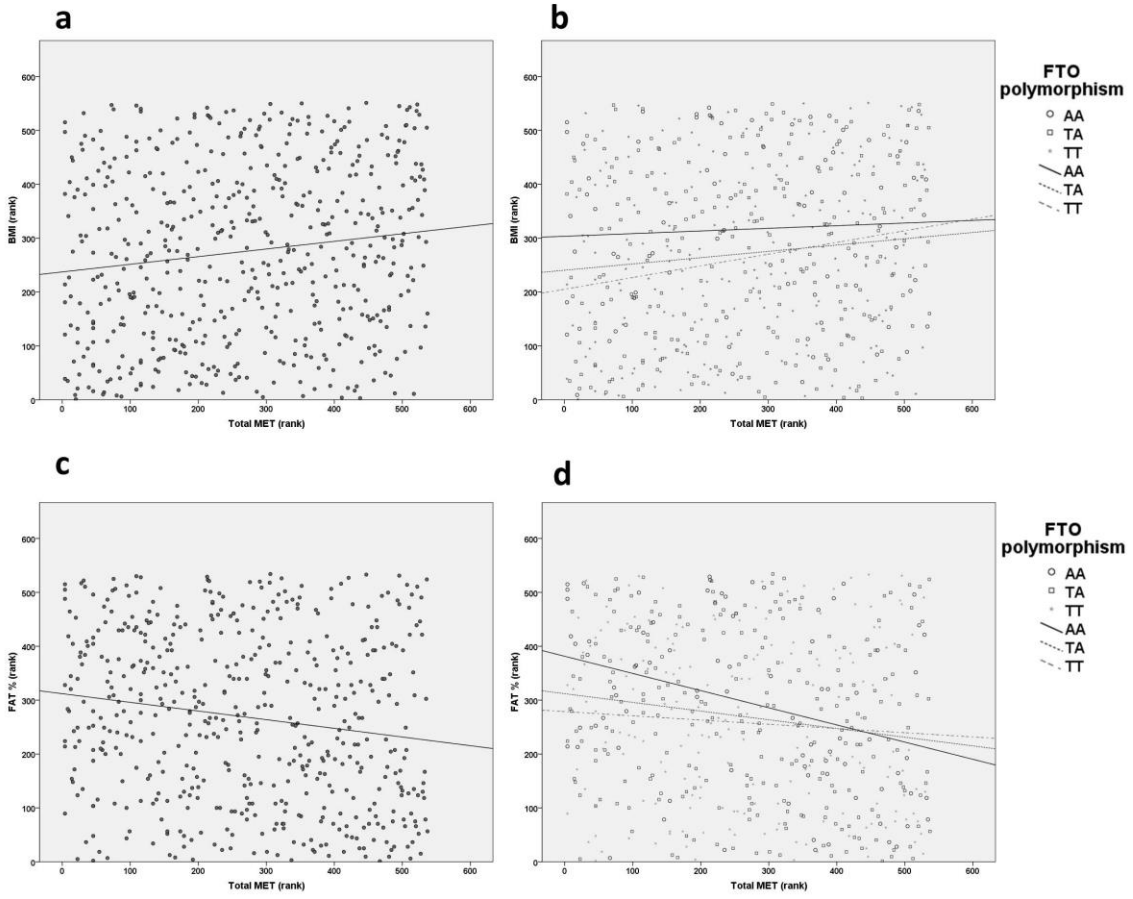

Table 1: Association of the FTO rs9939609 A/T polymorphism with quantitative traits in the studied population (n=550) based on linear regression models.

Trait	N	Model	β	SE	*P*
BMI (kg/m^2)	550	1	0.44	0.23	0.054
		2	0.46	0.22	**0.040**
FAT (%)	533	1	0.90	0.48	0.058
		2	0.67	0.31	**0.031**
Weight (kg)	550	1	0.61	0.79	0.436
		2	0.81	0.67	0.226
Height (m)	550	1	-0.75	0.55	0.174
		2	-0.57	0.38	0.137

Abbreviations: BMI, body mass index; FAT %, body *fat percentage*; N, number of analysed samples.
Table includes the effect sizes (regression coefficient beta, β) of the minor rs9939609 A-allele, standard error (SE) and P-values (asymptotic P-value) for quantitative traits, unadjusted (model 1) and adjusted for age and sex (model 2).
Significant results (*P*<0.05) are in bold and underlined.

Table 2: Association of FTO rs9939609 with the risk of overweight (including obese) for the total population and further split per sport practicing.

Groups	Genotypes	Normal N (%)	Overweight/Obese N (%)	OR (95% CI)*	*P**	*P***
Total sample (N = 550)	AA AT TT	59 (13.6) 204 (47.0) 171 (39.4)	25 (21.5) 51 (44.0) 40 (34.5)	1.32 (0.98-1.78)	0.064	**<u>0.034</u>**
Practicing sport (N=152)	AA AT TT	17 (14.8) 51 (44.3) 47 (40.9)	5 (13.5) 17 (46.0) 15 (40.5)	0.991 (0.57 -1.71)	0.97	0.84
Not practicing sport (N=398)	AA AT TT	42 (13.2) 153 (47.9) 124 (38.9)	20 (25.3) 34 (43.0) 25 (31.7)	1.54 (1.06 - 2.21)	**<u>0.02</u>**	**<u>0.007</u>**

Abbreviations: OR, odds ratio; CI, confidence interval. N, Number of genotyped samples.
* OR and *P*-value obtained by logistic regression under an additive model, and adjusted for age and sex.
** Asymptotic p-value calculated comparing normal *vs*. overweight and obese subjects, under a recessive model (genotypes AA compared to AT and TT).
Significant values (*P*<0.05) are in bold and underlined.