TV watching modifies obesity risk linked to the 27Glu polymorphism of ADRB2 gene in girls

Running title: TV and the 27Glu allele of ADRB2 gene in obese girls

Original article

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ABSTRACT

Objective

A matched case-control study was conducted in a Spanish children and adolescent population (5-18 years old), to assess the interaction between the Gln27Glu polymorphism of the ADRB2 and television (TV) watching on obesity risk.

Patients

Obese (n=165) and controls (n=165) matched by sex and age were recruited according to Spanish reference data.

Results

Using conditional logistic regression, we calculated the obesity risk linked to the polymorphism. A statistically significant association was found for 27Glu carriers allele girls (OR= 1.95; 95% CI= 1.02-3.70), but no association was apparent among boys. In the fully adjusted model, OR for obesity linked to the genotype Glu27Glu in the female population rose to 4.84 (95% CI =1.37-17.10).

Moreover, we found a significant negative interaction between hours of TV watching and the Gln27Glu polymorphism for obesity risk in girls. Surprisingly, among 27Glu carrier subjects, even girls with a low level of TV watching (<12.5 h/week) had a high obesity risk OR= 4.60 (95% CI= 1.01-20.02), which was not very different to the OR values for sedentary girls carrying the 27 Glu allele (TV watching more than 12.5 h/week, OR=6.05, 95% CI = 1.31-27.71).

Conclusion

A higher risk of obesity was found for girls carrying the 27Glu allele of the ADRB2 gene even when they spend less than 12.5h/week watching TV. In addition, our results suggest that the effect of sedentary lifestyle on obesity risk may depend on the genotype of the subject.

Keywords: ADRB2, Gln27Glu polymorphism, obesity risk, TV, children.

INTRODUCTION

There is some evidence that mutations of the ADRB2 gene may alter the lipolytic function contributing to fat accumulation. In adults, the Gln27Glu polymorphism of the ADRB2 gene has been associated with increased visceral fat (1-4) and *in vitro* studies indicate that this polymorphism seems to cause a resistance towards agonist-mediated downregulation (5). In an intervention study conducted by our group, we found that obese women homozygous for the 27Glu allele of the ADRB2 gene appear to have impaired exercise-stimulated lipolysis (1, 4). Moreover, in a case-control study, we showed that adult carriers of the 27Glu allele of the ADRB2 gene had higher BMI than non-carriers even when they were active during their leisure time (6).

Physical activity in children and adolescents seems to be declining, while their time spent in sedentary activities such as television (TV) watching is increasing (7). Time spent watching TV replaces more vigorous activities and exposes children to follow unhealthy habits about food intake so it is a risk factor for obesity (8, 9). This may explain why television viewing is a significant predictor of Body Mass Index (BMI) and overweight in childhood (10) and has been validated as a good index of sedentary behavior (11).

In this context, a matched case-control study was conducted to assess the interaction between the Gln27Glu polymorphism of the ADRB2 and TV viewing (as a proxy for sedentary lifestyles) in relation with obesity in a group of Spanish children and adolescents.

PATIENTS AND METHODS

The study population, recruited from the Pediatric Departments at the Virgen del Camino Hospital, University Hospital and some Primary Care Centers, comprised 330 Spanish children and adolescent. Cases (n=165) were subjects aged 5-18, with body mass index (BMI above the 97th percentile of the Spanish BMI reference data for age and gender (12)). Should we have used the International Obesity TaskForce (IOTF) standards, only 71% of Spanish obese children would have been classified as such (13, 14). Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrinopathy or serious intercurrent illness. Controls (n=165) were healthy subjects with a BMI below the 97th percentile of the same reference, matched with cases by sex and age (± 6 months). They were recruited when went to the Primary Care Centers for routine medical examination or to be vaccinated. The study was approved by the Ethics Committee of the University of Navarra and all parents and subjects over 12 years old provided written informed consent.

Anthropometric measurements were all collected in a medical environment by standard procedures. All the skinfolds were measured using a Holtain© skinfold caliper. Percentage of body fat was determined by bioelectrical impedance (TBF-300A Body Composition Analyzer/Scale, TANITA©, Tokyo, Japan). Blood samples were taken for the extraction of genomic DNA from leukocytes and the polymorphism was analyzed by PCR-RFLP (3).

Trained researchers conducted face-to-face interviews with participants and their parents with standardized procedures. A previously validated physical activity questionnaire was used (15). The questionnaire included 17 activities (sports and games) and nine response categories for frequency ranged from “never/almost never” to “six hours or more time per day”. A multiple of resting metabolic rate (MET score) was

assigned to each activity and an activity metabolic equivalent index (METs-h/week) was computed (16-18). This index represents the physical activity during the week for each participant. In addition, sedentary lifestyle was assessed through the number of hours spent watching TV or videos, during school days and on week-ends.

Statistical analyses

Descriptive values are given as mean and standard error of the mean (SEM). Univariate statistical analysis to compare the characteristics of each genotype group was performed using ANOVA and the Chi square test (for frequencies). When the data distribution was non-parametric, Kruskall-Wallis test was used. The p-value considered significant was <0.050 and between 0.050 and 0.100 was considered borderline significant.

Multivariate conditional logistic regression was used to estimate *odds ratios* (ORs) and to adjust for potential confounding factors. The results were adjusted for sex and age following the matched design. Four statistical models were built by introducing different variables: 1- the Gln27Glu polymorphism, 2- hours of TV watching, 3- physical activity and 4- a interaction product term (hours of TV watching x the Gln27Glu polymorphism). The same models were calculated to compare the homozygous genotype for the 27Glu allele (Glu27Glu) with the rest of the subjects (Gln27Gln and Glu27Gln carriers). The statistical analyses were done with SPSS (Statistical Package for the Social Sciences, Chicago, USA) 10.0.6 for Windows.

RESULTS

As expected, there was a significant difference in BMI between the obese subjects (27.8 [0.30] kg/m^2) and the controls (18.8 [0.20] kg/m^2; p<0.001). The frequencies of 27Glu allele of the ADRB2 gene were 0.439 in cases and 0.430 in controls, without

statistically significant differences between cases and controls either in boys or girls (Table 1). The genotype frequencies for these groups fulfilled the Hardy-Weinberg equilibrium (for girls, p=0.250; for boys, p=0.990).

Anthropometric and lifestyle data were similar in boys and girls, carriers or non carriers of the Gln27Glu polymorphism of the ADRB2 gene (Table 2). Using logistic regression analysis, we found that the Glu27Glu genotype of the ADRB2 gene was not associated with obesity in boys in the fully adjusted model (OR= 0.65; 95%CI= 0.19-2.19, Table 4). However, female subjects carriers of the 27Glu allele (25 homozygous: Glu27Glu and 93 heterozygous: Glu27Gln) had a significantly higher risk of obesity (OR=1.95; 95%CI=1.02-3.70) in the completely adjusted model (Table 3). When the ORs for obesity in girls were calculated for the presence of the 27Glu allele in homozygosis, the values were higher (OR=2.83; 95% CI=1.12-7.19). In the fully adjusted model, when physical activity (METs-h/week) and the usual time spent in watching TV were also taken into account and introduced in the model, the adjusted OR for obesity linked to the genotype Glu27Glu in the female population rose to 4.84 (95%CI=1.37-17.10).

An effect modification (interaction) for obesity risk linked to the Gln27Glu polymorphism of the ADRB2 gene was observed with hours of TV watching (p=0.023) after adjustment for physical activity (Fig. 1). In order to calculate different ORs for sedentary and non-sedentary subjects, we split the female population using the median of hours spent watching TV (12.5 hours per week, Fig. 2). In homozygous subjects for the wild-type genotype, the risk of obesity increased 7 times for sedentary (i.e. they spent >12.5 h/week watching TV, OR= 7.27, 95%CI=1.42-37.13) as compared to non-sedentary girls (<12.5 h/week of TV watching). Surprisingly, among 27Glu carrier subjects, even girls with a low level of TV watching (<12.5 h/week) had a high obesity

risk OR= 4.60 (95% CI= 1.01-20.02), which was not very different to the OR values for sedentary girls (TV watching more than 12.5 h/week, OR=6.05, 95% CI = 1.31-27.71).

DISCUSSION

Our results show that the Gln27Glu and Glu27Glu genotypes of the ADRB2 gene are associated with obesity in girls, but they do not appear to be related with obesity in males. A gender-dependent effect of the ADRB2 polymorphism has been previously reported in the literature (3, 6, 19, 20). This gender-dependent effect may be partially explained because the regulation of lipolysis differs markedly between men and women and may also be influenced by the ADRB2 polymorphism (2, 6).

A gene-physical activity interaction has been seen in different studies on energy expenditure regulatory genes (UCP3, ADRB3, ADRA2B, ADRB2), suggesting that the protective effect of physical activity for obesity development may depend on the individual genotype (21). For this reason, we analyzed the interaction between sedentary lifestyle and the Gln27Glu polymorphism of the ADRB2 using TV viewing as an index of a sedentary lifestyle for children and adolescents (11). In homozygous girls for the wild-type genotype, the relative risk of obesity proved to be directly related to the usual time spent in TV watching. However, carriers of the 27Glu allele of the ADRB2 gene had a high obesity risk independent of the time spent watching TV. Thus, a reduction in the usual time spent in TV watching may not be equally effective at preventing or treating obesity in these girls. These data are consistent with previous studies performed by our group and others suggesting that the Gln27Glu variant of the ADRB2 gene may decrease energy expenditure during physical activity, therefore leading to a resistance to weight loss in subjects with high to moderate levels of physical activity (1, 6, 22, 23). As our study is relatively small in size, it is not possible

to definitively rule out a type 1 error, although the matched design meant that less adjustment of variables is necessary and this increases the power of the study.

This is the first report studying the effect of the interaction between the Gln27Glu polymorphism of the ADRB2 gene and TV watching on obesity risk in a children and adolescent population, to our knowledge. The results suggest that carriers of the 27Glu allele of the ADRB2 gene may not benefit from a reduction in sedentary behaviors as much as the subjects who do not carry the polymorphism. Obesity is one of the most common chronic disorders in children and adolescents, so providing effective interventions is mandatory (24). However, more studies using bigger samples are needed to confirm these results. In this context, it is important to take into consideration these findings and to continue conducting research in order to design programs for keeping children physically active in order to prevent obesity.

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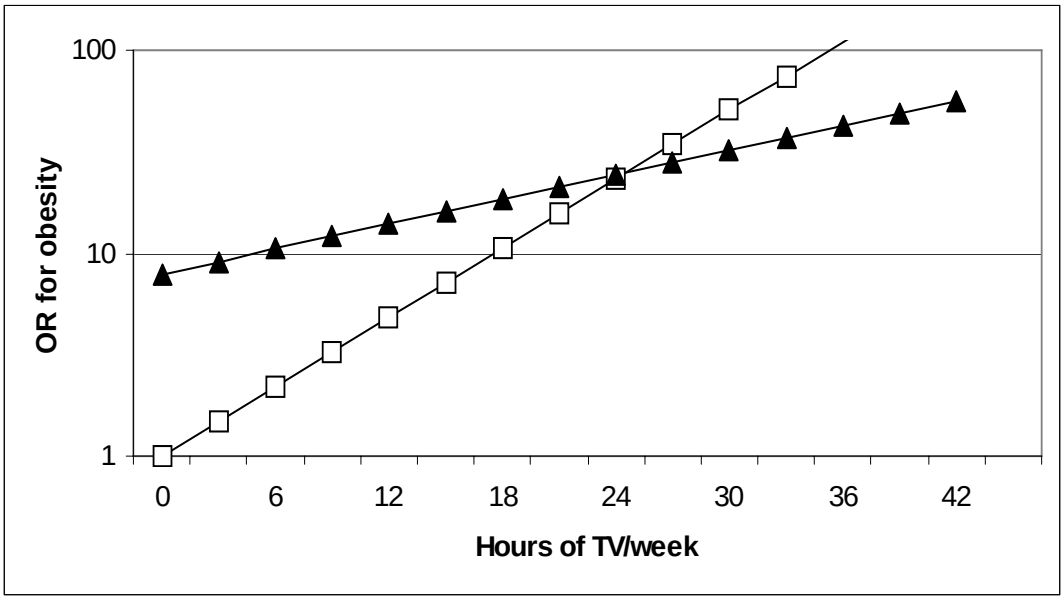
Figure legends

Figure 1. Change in the magnitude of the OR for obesity risk in girls depending on hours of TV watching according to the presence of the Glu27 allele of the ADRB2 gene (▲=carriers, □ = non-carriers).

Figure 2. Odds Ratio (OR) for obesity risk for groups of girls who watch TV more than 12.5h/week or less according to genotype (presence or absence of the 27Glu allele). ORs are adjusted for physical activity.

Figure 1



Girls	OR	95% CI	P
Gln27Glu genotype	7.92	1.89-33.19	*0.005*
Physical activity (METs-h/week)	0.92	0.88-0.96	*<0.001*
TV watching (h/week)	1.14	1.05-1.24	*0.003*
Interaction term (product = Gln27Glu X TV watching)	0.92	0.85-0.99	*0.023*

Figure 2

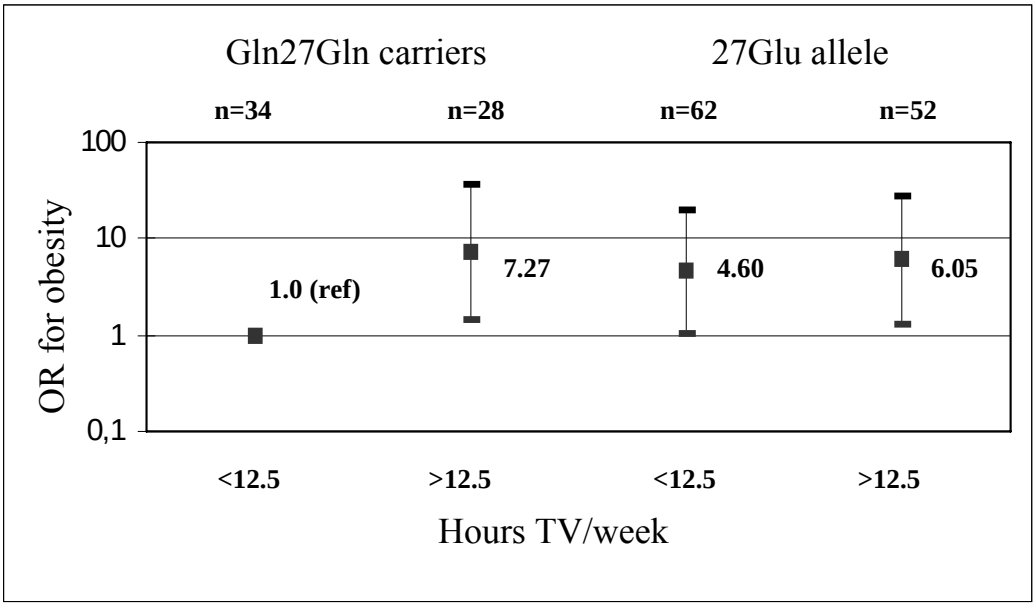

Gln27Gln carriers
27Glu allele
n=34
n=28
n=62
n=52
100
10
1
0,1
OR for obesity
1.0 (ref)
7.27
4.60
6.05
<12.5
>12.5
<12.5
>12.5
Hours TV/week

Table 1 Prevalence of the Gln27Glu polymorphism of the ADRB2 gene in a population of Spanish children and adolescents.

	All subjects			Boys			Girls		
notype	Obese (n=165)	Control (n=165)	p*	Obese (n=82)	Control (n=82)	p*	Obese (n=83)	Control (n=83)	p*
27Gln	51	49		29	23		22	26	
27Glu	83	90		40	40		43	50	
27Glu	31	26	0.683	13	19	0.403	18	7	0.058
elic frequency									
Gln	0.561	0.570		0.597	0.524		0.524	0.614	
Glu	0.439	0.430	0.814	0.403	0.476	0.394	0.476	0.386	0.096

*p-value by Chi-square test

Table 2 Anthropometric and lifestyle parameters in boys and girls according to the presence or absence of the Gln27Glu polymorphism of the ADRB2 gene.

Boys (n=164)	Gln27Gln (n=52)	Gln27Glu (n=80)	Glu27Glu (n=32)	p†
Age (years)	12.3 (0.30)	11.6 (0.27)	12.0 (0.46)	0.261
BMI (kg/m^2)	24.8 (0.85)	23.0 (0.66)	22.4 (0.99)	0.146
BMI z-score	2.56 (0.35)	1.98 (0.26)	1.59 (0.38)	0.189
% Body fat	24.7 (1.69)	24.6 (1.50)	20.4 (1.95)	0.332
Triceps skinfold (mm)	20.0 (1.17)	19.8 (0.94)	19.4 (1.37)	0.851
Subscapular skinfold (mm)	20.7 (1.96)	17.0 (1.21)	15.1 (1.64)	0.196
Height/Waist perimeter (m/cm)	0.51 (0.01)	0.51(0.01)	0.50 (0.02)	0.854
METs-h/week	34.9 (2.91)	34.4 (2.70)	34.4 (3.18)	0.722
TV watching (h/week)	12.1 (1.17)	14.2 (0.99)	15.6 (1.79)	0.189
Girls (n=166)	**Gln27Gln (n=48)**	**Gln27Glu (n=93)**	**Glu27Glu (n=25)**	**p†**
Age (years)	11.8 (0.48)	11.1 (0.28)	10.8 (0.60)	0.293
BMI (kg/m^2)	23.6 (0.98)	22.5 (0.51)	24.6 (1.34)	0.336
BMI z-score	1.87 (0.34)	1.64 (0.19)	2.56 (0.46)	0.182
% Body fat	30.9 (1.46)	28.9 (1.12)	33.7 (1.94)	0.120
Triceps skinfold (mm)	20.7 (1.12)	21.3 (0.73)	22.9 (1.18)	0.452
Subscapular skinfold (mm)	19.5 (1.55)	18.5 (0.98)	21.3 (1.74)	0.329
Height/Waist perimeter (m/cm)	0.50 (0.02)	0.49 (0.01)	0.51(0.02)	0.728
METs-h/week	22.4 (1.97)	21.1 (1.42)	20.1 (2.81)	0.592
TV watching (h/week)	16.9 (1.36)	13.3 (0.82)	14.3 (1.72)	0.072

Mean (SEM). †p-value by Kruskall-Wallis or ANOVA test.

Table 3 OR of obesity (95% CI) associated with the polymorphism Gln27Glu of the ADRB2 gene in girls. The reference categories were 27Gln carriers in case of Glu27Glu genotype and Gln27Gln carriers in case of Gln27Glu plus Glu27Glu genotypes. Estimates are adjusted for the other variables shown in the table for in each statistical model.

Girls	OR	95%CI	P
Gln27Glu and Glu27Glu genotypes			
Model 1			
Gln27Glu and Glu27Glu	1.53	0.94-2.47	0.084
Model 2			
Gln27Glu and Glu27Glu	1.68	1.00-2.84	0.051
TV watching (h/week)	1.05	1.01-1.09	*0.023*
Model 3			
Gln27Glu and Glu27Glu	1.95	1.02-3.70	*0.042*
Physical activity (METs-h/week)	0.93	0.90-0.97	*<0.001*
TV watching (h/week)	1.06	1.01-1.11	*0.026*
Glu27Glu genotype			
Model 1			
Glu27Glu genotype	2.83	1.12-7.19	*0.028*
Model 2			
Glu27Glu genotype	3.58	1.32-9.70	*0.012*
TV watching (h/week)	1.05	1.01-1.09	*0.019*
Model 3			
Glu27Glu genotype	4.84	1.37-17.10	*0.014*
Physical activity (METs-h/week)	0.93	0.90-0.97	*<0.001*
TV watching (h/week)	1.06	1.01-1.11	*0.024*

Table 4 OR of obesity (95% CI) associated with the polymorphism Gln27Glu of the ADRB2 gene in boys. The reference categories were 27Gln carriers in case of Glu27Glu genotype and Gln27Gln carriers in case of Gln27Glu and Glu27Glu genotypes. Estimates are adjusted for the other variables shown in the table for in each statistical model.

Boys	OR	95%CI	P
Gln27Glu and Glu27Glu genotypes			
Model 1			
Gln27Glu and Glu27Glu	0.71	0.44-1.14	0.162
Model 2			
Gln27Glu and Glu27Glu	0.74	0.45-1.20	0.229
TV watching (h/week)	1.00	0.96-1.04	0.951
Model 3			
Gln27Glu and Glu27Glu	0.74	0.39-1.38	0.344
Physical activity (METs-h/week)	0.93	0.89-0.96	*<0.001*
TV watching (h/week)	0.99	0.94-1.05	0.972
Glu27Glu genotype			
Model 1			
Glu27Glu genotype	0.57	0.24-1.36	0.207
Model 2			
Glu27Glu genotype	0.72	0.29-1.81	0.495
TV watching (h/week)	1.00	0.96-1.04	0.988
Model 3			
Glu27Glu genotype	0.65	0.19-2.19	0.494
Physical activity (METs-h/week)	0.93	0.90-0.96	*<0.001*
TV watching (h/week)	0.99	0.94-1.05	0.969