The lactase persistence -13910C>T polymorphism shows indication of association with abdominal obesity among Portuguese children

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Running title: Association between -13910C>T and abdominal obesity

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ABSTRACT

Aim: The -13910C>T single nucleotide polymorphism located upstream of the lactase gene (*LCT*) was found tightly associated with lactase persistence in European populations. Recently, it was also associated with body mass index (BMI) and obesity in European adults. The aim of this study was to test the association of -13910C>T polymorphism, with obesity-related traits and risk of obesity in children.

Methods: We genotyped 580 Portuguese children (6-12 years-old) for the -13910C>T polymorphism using TaqMan probes by real-time PCR. Anthropometric measurements were assessed in all children. Obesity was defined according to the IOTF cut-offs and abdominal obesity using the sex and age-specific ≥90th waist circumference percentile.

Results: We found indication for an association between the -13910*T allele and children abdominal obesity (OR =1.41; 95% CI: 1.03-1.94; *p*=0.030). Under the dominant model, the indicative association was observed between the *LCT* -13910 CT/TT genotypes and abdominal obesity, remaining significant after adjustment for age and gender (OR =1.65; 95% CI: 1.04-2.60; *p*=0.029). No association was detected with the risk of obesity (*p*=0.350).

Conclusion: Our results suggest that the -13910C>T polymorphism may predispose to abdominal obesity in Portuguese children. The association with BMI or obesity risk, previously observed in adults, was not confirmed.

Keywords: Abdominal Obesity, Waist circumference, BMI, Portuguese children, *LCT* -13910C>T polymorphism.

Key notes:

- The -13910C>T polymorphism, located upstream of the lactase gene and tightly associated with LP in European populations, was recently associated with BMI and obesity in European adults.

- We tested the association of -13910C>T polymorphism with obesity-related traits and risk of obesity in Portuguese children.
- We found indication for an association between the LP -13910*T allele and children abdominal obesity. No association was detected with obesity risk.

Abbreviations

BMI – Body Mass Index
DNA - Deoxyribonucleic Acid
IOTF – International Obesity Task Force
LCT – Lactase Gene
LN – Lactase Persistence
LNP – Lactase Non-Persistence
PCR - Polymerase Chain Reaction
SNP- Single Nucleotide Polymorphism

INTRODUCTION

The lactase gene (*LCT*; MIM 603202, chromosome 2q21) was recently reported as a new candidate related with BMI and obesity in adults of European origin. Three independent studies reported a strong association of the -13910C>T (rs4988235) single nucleotide polymorphism (SNP), located ~14kb upstream from the *LCT* coding region, with BMI and obesity: the -13910*T allele carriers (CT and TT genotypes) had higher weight, BMI and risk of obesity (1-3).

All newborns display an adequate expression of the lactase enzyme that decline significantly in quantity following weaning, and this condition is apparently the major reason for avoiding milk in diet (4). However, substantial numbers of individuals maintain the ability to digest milk and other dairy products into adulthood. This lactase persistence (LP) phenotype is an autosomal dominant condition that reaches its highest values in north-western Europe (80-90%), declining to the south and east (~50%) (5,6). The lactase non-persistence (LNP) is considered the ancestral condition in humans and these individuals are unable to digest

significant amounts of lactose, suffering from adverse unspecific abdominal symptoms, including bloating, abdominal pain and diarrhoea, after ingestion of milk (7). In European populations the -13910C>T SNP was found tightly associated with the persistence of the lactase enzyme in adulthood: TT or TC individuals possess sufficient enzyme activity in intestinal cells to be classified as LP, and individuals carrying the CC genotype are classified as LNP (8). The prevalence of the -13910*T allele vary across Europe, reaching 70-80% in Northern European populations, and 5-10% in Southern European populations from Greece and Italy (9). In Northern Portugal the -13910*T allele frequency was estimated 37.0% (10).

Until now, the genetic background of *LCT* -13910C>T polymorphism in obesity has not yet been examined among children of European descent. Thus, the aim of this study was to test the association between *LCT* -13910C>T polymorphism and obesity or obesity-related traits in a sample of Portuguese children.

MATERIAL AND METHODS

Subjects and measures

The study comprised 580 children with Portuguese ancestry (6-12 years old) randomly selected from several public schools in a Northern-Central region of Portugal located between *Mondego* and *Douro* rivers. Samples were collected from three geographic areas: municipalities of Coimbra (*n*=266), Vale de Cambra (*n*=147) and Guarda/Covilhã (*n*=167).

Anthropometric measurements were assessed in all children. Children were distributed in three groups: 140 with obesity, 233 with overweight and 207 with normal weight. The definition of overweight and obesity was obtained using the International Obesity Task Force (IOTF) cut-offs (11), resulting from the BMI in adult's cut-points of 25 kg/m^2 and 30 kg/m^2, respectively. Abdominal obesity was defined using the sex and age-specific $\geq 90^{th}$ waist circumference percentile (12).

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 guidelines of the University of Coimbra. Written informed consent was previously obtained from all the children´s parents.

Genotyping

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

The -13910C>T polymorphism was genotyped using TaqMan® probes for real-time PCR on a MiniOpticon instrument (Bio-Rad, Hercules, CA, USA) using primers and labelled probes previously reported (13). The PCR amplification was carried out in 20 µl of a total reaction volume containing 1.5 µl (~40 ng) of DNA, 0.4 µM primers, 0.2 µM probes in 1x of SsoFast™ Probes Supermix (Bio-Rad, Hercules, CA, USA). PCR conditions were an initial denature step at 95°C for 10 minutes, followed by 40 cycles of 1 minute at 60°C and 15 seconds at 95°C. To assess genotyping reproducibility, a random 10% selection of samples was re-genotyped with 100% concordance.

Statistical analysis

Allelic and genotypic frequencies of the -13910C>T polymorphism were estimated by direct counting. Hardy-Weinberg equilibrium probability value, heterozygosity and exact *p* values for population differentiation (14) were achieved using the software package Arlequin, v.3.11 (http://cmpg.unibe.ch/software/arlequin3/) (15).

In statistical analyses we follow a dominant model, and subjects with CT and TT genotypes were grouped and compared with CC subjects. Student *t*-test was used to compare means of obesity-related traits between genotypes, and a general linear model was used to adjust for

age and gender. Logistic regression was used to estimate *p* values, odds ratio (OR), and 95% confidence intervals (CI), assessing the association of -13910C>T polymorphism with risk of obesity and abdominal obesity. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version. 19.0; Armonk, NY).

RESULTS

The anthropometric characteristics of the study subjects distributed by phenotype are shown in Table 1. The *LCT* -13910C>T genotype distributions regarding whole sample (n=580) were: CC 42.8%, CT 44.8% and TT 12.4%. Individual LP -13910*T allele frequency was 34.8%. The similar allele frequencies across the three sampled subgroups (-13910*T allele frequency values of 34.6%, 32.3% and 37.4% in Coimbra, Vale de Cambra and Guarda/Covilhã municipalities, respectively) indicate absence of population stratification for the -13910C>T polymorphism (p=0.466 on the exact test of sample differentiation). No deviations from the Hardy-Weinberg equilibrium were observed in any population subgroup, neither in the whole sample (p>0.05).

Testing the association with anthropometric variables, we found no association between the -13910C>T polymorphism and obesity-related traits, as BMI, weight or waist circumference (p>0.05 for all traits) (Table 2).

Logistic regression analysis was used to test the association of the -13910C>T polymorphism with the risk of obesity and abdominal obesity (Table 3). We observe indication of an association between the LP -13910*T allele and abdominal obesity (OR = 1.41; 95% CI: 1.03-1.94; p = 0.030). In a dominant model of genetic effect, the -13910 CT/TT genotypes show also indication of an association with abdominal obesity (unadjusted OR = 1.64; 95% CI: 1.04-2.62; p = 0.034), remaining significant after adjustment for age and gender (OR = 1.65; 95% CI: 1.04-2.60; p = 0.029). No association was found between the -13910 CT/TT genotypes and

obesity (OR = 1.24; 95% CI: 0.79-1.92; *p* = 0.350) or overweight (OR = 0.86; 95% CI: 0.59-1.28; *p* = 0.769).

DISCUSSION

Several genetic polymorphisms have been reported to be associated with obesity or obesity-related phenotypes both in children and adults (16), which is a growing problem worldwide including Portugal (17). To better understand the genetic basis of obesity it is important to replicate these results in different populations across the world. Recently it was reported that *LCT* -13910C>T polymorphism is strongly associated with BMI and obesity in European adults by three studies that found adult's carriers of the -13910 CC genotype with lower weight and BMI (1-3).

There are some controversial data regarding effects of dairy products intake on body weight and fat (18). Several studies in adults support for an increase in body weight associated with dairy products intake (19-21). One possible explanation for this association could be that the extension of consumption of dairy products in daily diet, often high in energy content, potentially increase calorie intake in adulthood (21). In children, relationship between dairy products consumption and weight/body composition indicate either a beneficial or a neutral effect (22,23). However, no studies were performed to see if individual *LCT* genetic profiles influence the relationship between dairy products consumption and body weight.

We conducted a population study to test whether the -13910C>T polymorphism, tightly associated with LP in individuals of European descent, was associated with obesity and/or obesity-related traits in Portuguese children. The exact test of sample differentiation based on allele frequencies showed no significant differences between the three study geographic areas of Coimbra, Vale de Cambra and Guarda/Covilhã, excluding population substructure and possible bias in association signals for the -13910C>T polymorphism, a locus that was shown to be prone to population stratification (24). We found indication of an association between the

LP -13910 CT/TT genotypes and abdominal obesity (OR =1.65; *p*=0.032), however, we did not find evidence for the association of the -13910C>T polymorphism with the children risk of obesity or other anthropometric measurements.

Considering previous studies showing that obesity risk in adulthood is significantly higher in T-allele carriers (TT and CT genotypes) than in CC subjects (1-3), our findings suggest that, by a continuous intake of rich fat dairy products, individuals with -13910 CT/TT genotypes could have a more predisposition to develop obesity into adulthood than individuals with -13910 CC genotype, associated with adult hypolactasia.

In conclusion, this study suggests that LP -13910 CT/TT genotypes may predispose to abdominal obesity in Portuguese children. Association of the -13910C>T polymorphism with BMI or risk of obesity, previously observed in adults, was not confirmed in children. Further studies are needed i) to replicate the present results in children from other populations; ii) to see whether individual *LCT* genetic profiles influence the relationship between dairy products consumption and obesity-related traits.

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Conflict of interests

The authors have no conflict of interest.

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Table 1 Body size and genetic characteristics of the children participants according to phenotype.

Characteristics	Overall	Phenotype		
		Normal	Overweight	Obese
n	580	207	233	140
Age (years)	9.0 ± 1.7	8.6 ± 1.6	9.6 ± 1.6	9.0 ± 1.8
Girls (%)	51.9	54.1	48.1	55.0
Height (cm)	136.1 ± 11.8	130.8 ± 11.1	139.8 ± 11.3	137.8 ± 10.8
Weight (kg)	37.4 ± 11.6	27.9 ± 6.6	40.5 ± 9.5	45.9 ± 11.2
BMI (kg/m^2)	19.7 ± 3.5	16.1 ± 1.4	20.4 ± 1.8	23.8 ± 2.6
BMI Z-score	0.95 ± 0.9	-0.11 ± 0.8	1.28 ± 0.24	1.98 ± 0.2
Waist circumference (cm)	67.4 ± 7.8	60.4 ± 4.4	69.1 ± 5.4	74.9 ± 6.6
Hip circumference (cm)	79.2 ± 10.5	70.4 ± 6.3	82.3 ± 8.3	87.1 ± 9.5
Waist-to-Height Ratio	0.86 ± 0.06	0.86 ± 0.06	0.84 ± 0.06	0.86 ± 0.05

Abbreviations: *n*, total subjects; BMI, body mass index; BMI Z-score, body mass index standard deviation.

Phenotype was defined using the International Obesity Task Force (IOTF) cut-offs.

Data are presented as mean ± standard deviation.

Table 2 The *LCT* -13910C>T polymorphism genotype and allelic distribution among municipalities.

Municipalities	*n*	Genotype distribution, % (*n*)			Allele frequencies		*p*	He
		CC	CT	TT	C	T		
Coimbra	266	43.6 (116)	43.6 (116)	12.8 (34)	0.654	0.346	0.589	0.453
Vale de Cambra	147	45.6 (67)	44.2 (65)	10.2 (15)	0.677	0.323	1.000	0.439
Guarda/Covilhã	167	38.9 (65)	47.3 (79)	13.8 (23)	0.626	0.374	1.000	0.469
Total	580	42.8 (248)	44.8 (260)	12.4 (72)	0.652	0.348	0.788	0.454

Abbreviations: *n*, total subjects; *p*, exact *p*-value for the Hardy-Weinberg equilibrium (*p* significant <0.05); He, expected heterozygosity.

Table 3 Comparison of anthropometric parameters among different genotypes of *LCT* -13910C>T polymorphism.

	LCT -13910C>T genotypes		*p*-value[a]	*p*-value[b]
	CC (*n*=248)	CT/TT (*n*=332)		
Age (years)	9.0 ± 1.7	9.1 ± 1.7	0.873	
Height (cm)	135.3 ± 11.8	136.8 ± 11.7	0.870	0.237
Weight (kg)	36.5 ± 11.4	37.9 ± 11.7	0.420	0.562
BMI (kg/m^2)	19.5 ± 3.4	19.8 ± 3.6	0.244	0.268
BMI Z-score	0.91 ± 0.9	0.98 ± 0.9	0.346	0.630
Waist circumference (cm)	67.0 ± 7.7	67.7 ± 8.0	0.626	0.754
Hip circumference (cm)	78.6 ± 10.6	79.6 ± 10.4	0.857	0.324
Waist-to-Height Ratio	0.85 ± 0.04	0.86 ± 0.05	0.350	0.866

Data are presented as mean ± standard deviation.

p-value[a] unadjusted.

p-value[b] adjusted for age and gender. (*p*-value significant <0.05).

aTable 4 Associations of *LCT* -13910C>T polymorphism with risk of obesity and abdominal obesity [OR (95% CI)].

	Genotype distribution, % (*n*)					OR (95% CI)		
	Normal *n*=207	Overweight *n*=233	Obese *n*=140	Gluteofemoral *n*=483	Ab obesity *n*=97	Normal *vs.* Obese	Normal *vs.* Overweight	Gluteofemoral *vs.* Ab obesity
Dominant Model								
CC	43.0 (89)	45.5 (106)	37.9 (53)	44.7 (216)	33.0 (32)	1	1	1
CT/TT	57.0 (118)	54.5 (127)	62.1 (87)	55.3 (267)	67.0 (65)	1.24 (0.79-1.92) *p*=0.350	0.86 (0.59-1.28) *p*=0.769	1.65 (1.04-2.60) *p*=**0.032**
Allelic Model								
C	65.6 (273)	65.7 (307)	61.7 (174)	66.4 (641)	58.2 (113)	1	1	1
T	34.4 (141)	34.3 (159)	38.3 (106)	33.6 (325)	41.8 (81)	1.17 (0.86-1.62) *p*=0.305	1.00 (0.76-1.32) *p*= 1.00	1.41 (1.03-1.94) *p*=**0.029**

p-value under the dominant and allelic model was adjusted for age and gender (*p*-value significant <0.05 in bold).

Overweight and obesity was defined using the International Obesity Task Force (IOTF) cut-offs.

Abbreviations: Ab obesity; abdominal obesity defined using the sex and age-specific ≥90th waist circumference percentile. Gluteofemoral, children under the <90th waist circumference percentile. OR, odds ratio; CI, confidence interval.