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EFFECT OF THE PEROXISOME PROLIFERATORS-ACTIVATED RECEPTOR (PPAR) GAMMA 3 GENE ON BMI IN 1,210 SCHOOL STUDENTS FROM MORELOS, MEXICO [*]

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Little research has been undertaken on risk factors for obesity in young people in Latin America, including Mexico, despite the fact that obesity constitutes the number one public health problem in Mexico. Our objective was to investigate the effect of the Peroxisome proliferators-activated receptor (PPAR)_3 gene on BMI measured among adolescents collected from a cohort study originally designed for epidemiological studies. METHODS: Blood samples and anthropometric measurements were collected from 1,210 out of 13,294 public school students of both sexes, aged 11-24 years in Morelos, Mexico. In this study, we genotyped 7 selected SNPs of the PPAR_ transcript variant 3 (including Pro12Ala) in a group of unrelated 717 males and 493 females (age range 11-24), including 3 SNPs located in the 5' untranslated region. These 7 SNPs were selected by the tagging algorithm implemented in the program haploview to scan the whole gene. We tested each of the 7 SNPs individually for association with the body mass index (BMI), and two SNPs (rs2938392 and rs1175542) revealed significant associations with BMI (p-value=0.008 and 0.029, respectively). The SNP rs2938392 is roughly 41.5 Kb from rs1801282 (Pro12Ala in PPAR_2). Furthermore, we examined the association between haplotypes built from 7 SNPs and BMI using a score statistic implemented in the program haplo.stats. While the permutation based global p-value was 0.544, one individual haplotype with a frequency of 0.279 gave a p-value of 0.089

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(permutation based). However, when the analyses were conducted in males only, the permutation based global p-value was 0.055 and one individual haplotype with a frequency of 0.28 gave a significant p-value of 0.013.

1. Introduction

The goal of this study was to investigate the effect of the Peroxisome proliferators-activated receptor (PPAR)_3 gene on Body Mass Index (BMI) measured among Mexican adolescents. Although it has been established that both PPAR_2 and PPAR_3 are expressed uniquely in colon and adipocyte tissue and their potential role in the metabolic disorders such as obesity and type 2 diabetes has been suggested [1], the reported effects of the Pro12Ala polymorphism on susceptibility for obesity have been inconsistent [2]. The detailed findings from all positive or negative studies are given in Table 1.

Table 1 A summary of Pro12Ala findings in different studies

Ref	Sample size	Ethnicity	P-value
[3]	517 being lean-to-moderately obese	Caucasian	0.01
[3]	169 very obese	Caucasian	<0.001
[4]	333 non-diabetic	Scandinavian	0.027
[4]	973 non-diabetic	Scandinavian	0.015
[5]	215 men	Asian	0.65
[6]	296 extremely obese	Caucasian	>0.05
[6]	130 underweight	Caucasian	>0.05
[7]	752 obese	Caucasian	0.008
[7]	869 non-obese	Caucasian	0.005
[8]	141 obese	Scandinavian	0.011
[9]	131 diabetic	Caucasian	0.8
[9]	312 normoglycemic	Caucasian	0.9
[10]	1025 diabetic	Caucasian	>0.05
[10]	310 with normal BMI	Caucasian	>0.05
[11]	108 non-diabetic	Caucasian	0.67
[11]	19 overweight	Caucasian	0.71
[12]	295 non-diabetic non-obese	Caucasian	>0.05
[12]	372 morbidly obese	Caucasian	>0.05
[12]	402 diabetic	Caucasian	>0.05
[13]	541 non-diabetic	Asian	0.15
[13]	415 diabetic subjects	Asian	0.10
[14]	165 obese	Caucasian	0.017
[15]	229	Asian	>0.05
[16]	921	Caucasian	0.011
[17]	476	Scandinavian	0.3
[18]	675 men	Caucasian	0.64

ID	Sample size	Ethnicity	P-value
[19]	228 with normal BMI	Scandinavian	0.070
[19]	217 with dyslipidemia	Scandinavian	0.034
[19]	649 without dyslipidemia	Scandinavian	0.080
[20]	280 with normal BMI	Caucasian	>0.05
[20]	95 obese	Caucasian	0.32
[20]	42 young obese	Caucasian	<0.05
[21]	619	Caucasian	0.035
[22]	453 from 10 families	Mexican	>0.05
[23]	2201 diabetics	Asian	0.881
[23]	1212 with normal BMI	Asian	0.846
[24]	292 obese	Caucasian	0.89
[24]	371 lean	Caucasian	0.47
[25]	259 men	Caucasian	0.554
[25]	333 women	Caucasian	0.678
[26]	124 non-diabetics	Caucasian	0.31
[27]	2245 non-diabetics	Scandinavian	>0.05
[28]	1107 diabetics	Caucasian	0.3
[29]	438	Caucasian	>0.05
[30]	478 men	Asian	>0.05
[30]	117 women	Asian	>0.05
[31]	145 obese	Caucasian	>0.05
[31]	317 non-obese	Caucasian	>0.05
[32]	210 monozygotic twins	Scandinavian	0.09
[32]	344 dizygotic twins	Scandinavian	>0.05
[33]	720	Caucasian	0.005
[34]	253 with low physical activity level	Caucasian	>0.05
[34]	253 with high physical activity level	Caucasian	p<0.05
[35]	420 diabetic	Asian	0.566
[35]	538 with impaired glucose tolerance	Asian	0.875
[35]	3080 with normal BMI	Asian	0.037

Few studies have investigated the association between PPAR_ gene and BMI in the Mexican population. The most recent report was from Hsueh *et al*. 2001 [22], based on the study of 453 subjects comprising of 10 pedigrees. However, no significant effect was found in their study.

While most of previous studies have focused on the effect of Pro12Ala or PPAR_2, we have chosen to study PPAR_3, which contains the region of PPAR_2, but also includes a 5' untranslated region of the PPAR_ gene. Interestingly, the expression of PPAR_3 is directed by an independent promoter, and to date, at least three promoters in the upstream region of PPAR_3 have been identified through molecular studies [1]. We analyzed seven

tagging SNPs spanning 89.5kb of PPAR_3 gene region, and four of them were located in the long 5'-end untranslated region. Our motivation is to examine the effect of the haplotypes that represent the PPAR_3 genes, rather than focusing on Pro12Ala itself.

2. Methods

2.1. *Study Population*

This study builds on a parent cohort study carried out by Lazcano-Ponce et al. (2003). The parent study was the 1998-1999 baseline measurement of a cohort study of 13,293 students at public junior high and high schools and a state university in the central Mexican State of Morelos. The Research Ethics committee of the National Institute of Public Health approved the study Protocol for epidemiological studies. Further, The IRB review board of Johns Hopkins Bloomberg School of Public Health approved the current study which uses 1,270 anonymous DNA samples out of 13,293 Mexican students to test for association between BMI and SNPs residing in the PPARγ3 gene. Table 2 gives information on age and gender in this study population.

Table 2 Characteristics of the study group subjects

Variable	Male	Female	Whole population
No. of Subjects	717	493	1210
Age	15.56±2.67	15.84±2.93	15.67±2.78
BMI	23.76±5.76	23.45±5.61	23.63±5.7

2.2. *DNA extraction and Genotyping*

DNA extraction was performed using the Gentra DNA extraction kit following the manufacturer's suggestion. Out of 1,270 buffy coat samples, 1,210 samples gave sufficient DNA for this study. TaqMan, developed by Applied Biosystems (ABI), is an efficient system and genotyping was performed at the core genotyping facility at Johns Hopkins University.

The PCRs were conducted with both primers and probes added and only end point products were read. The Hydra and Biomek FX was used to dispense DNA samples and set up PCRs, respectively. The PCR was conducted in two 9700 thermocyclers each equipped with a dual 384-well blocks. Then the end point products were scored using the 7900HT. In each 384-well plate, two reference samples were included for quality control. The primers and probes were designed using Primer Express (ABI). The probes were labeled with two fluorescent dyes, one as an indicator and the other as a quencher. Two probes

were synthesized for each locus, each labeled with two different dyes as indicators, respectively.

2.3. *SNP tagging*

We used the haploview program, http://www.broad.mit.edu/mpg/haploview/ [37] to compute the pair-wise LD measure D'. Haploview estimates the maximum-likelihood values of the 4 gamete frequencies, from which D' can be calculated. For these calculations we included members of our melanoma families as well as 350 individuals with BMI less than 25. The SNP haplotype tagging strategy (*htSNP*) allowed us to identify regions of strong LD using the Gabriel *et al* block definition [38], and kept every inter-block SNP plus the single SNP within each block with the highest minor allele frequency (MAF). The LD structure of these 7 selected SNPs is presented in Figure 1. The marker rs1801282 represents Pro12Ala in PPAR_2.

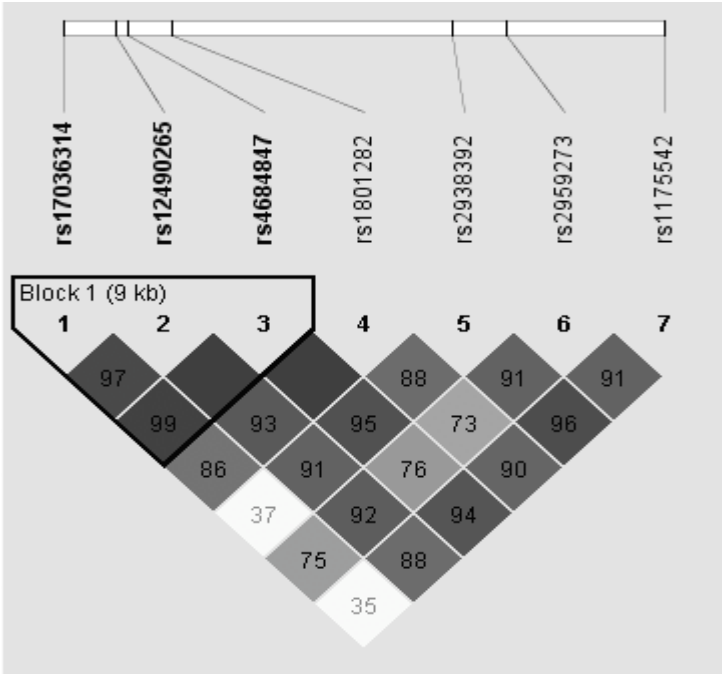

Figure 1 LD structure of the selected SNPs in PPAR_3. The numbers within each block indicate the pair –wise D' as a percentage. The higher LD linkages are shown in the darker color, while the first three SNPs constitute an LD block spanning 9kb of gene region. Moreover, all of these three SNPs are located in the 5'-untranslated region of PPAR_3.

2.4. *Statistical analysis*

Hardy-Weinberg proportions were tested among individuals with BMI less than 25 using the Pearson chi-square test. Pair-wise linkage disequilibrium was evaluated by two linkage disequilibrium parameters, Lewontin's D′ [39] and r^2 [40], which were both calculated in haploview (data not shown).

2.4.1. *Single SNP analysis*

Single SNP analyses were conducted using the linear regression procedure implemented in STATA version 9. The SNP effects were analyzed using three models which were, respectively: additive, dominant and recessive models. In the additive model, the wild type genotype, the homozygote consisting of the most common allele in the population, was treated as the baseline and coded as 1, while the heterozygote was coded as 2 and the other homozygote was coded 3. In the dominant model, the wild type homozygote was coded by 1 as baseline and the heterozygote and the other homozygote were both coded 2. Moreover, in the recessive model, both of the wild type homozygote and heterozygote were treated as baseline with the rare homozygote coded 2.

2.4.2. *Haplotype analysis*

All haplotype frequencies were estimated using the expectation maximization (EM) algorithm in haplo.stats in the R programming language (http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm). Schaid et al [41] developed a score statistic that can be used to test the statistical association between haplotypes and different types of human traits, including binary and quantitative traits. This method also allows adjustment for non-genetic factors. In this analysis, we used haplo.stats to compute the global score statistic (which allows us to test the significance of association considering all haplotypes) and obtain a global p-value and the haplotype specific statistic (which allows us to compare each haplotype with a selected common haplotype). All haplotypes with a frequency less than 1% were dropped from the score test in order to reduce the degrees of freedom. Another advantage of Schaid's method is to compute empirical p-values using simulation when the haplotype data is sparse. The empirical p-values are computed by repeatedly first permuting the genotypes among the subjects and then computing the score statistics.

3. Result

Hardy-Weinberg proportions were tested among individuals with BMI less than 25 using the Pearson chi-square test and no SNPs showed deviation from Hardy Weinberg equilibrium. The minor allele frequencies are 0.479, 0.130, 0.134,

0.143, 0.445, 0.151 and 0.441 for SNPs rs17036314, rs12490265, rs4684847, rs1801282, rs2938392, rs2959273, rs1175542, respectively.

Linear regression analysis of single SNP revealed two SNPs associated with BMI with significant p-values reported in Table 3 (non-adjusted for multiple testing).

Table 3. Significant associations revealed by single SNP analysis and the body mass index (BMI)

SNP ID	Location	Population	Model	Coefficient	P-value
rs2938392	Second intron	Whole	Dominant	1.0866	0.008
rs2938392	Second intron	Male	Dominant	1.3818	0.009
rs1175542	Fifth intron	Whole	Recessive	0.898	0.029
rs1175542	Fifth intron	Male	Recessive	1.171	0.029

In addition, we used a sliding window technique to fine map any potential signals within the larger haplotype using haplo.stats [41]. The size of the window varied from 3-4 SNPs in order to provide a comprehensive assessment of the haplotype subsets within the gene. Permutation based p-values were summarized in Table 4 for significant SNP combinations.

Table 4. Haplotypes revealing significant associations with BMI using 1,210 Mexican samples, p-value(sim) indicated the p-values based on permutation.

Haplotypes	Frequency	P-value	P-value (sim)
rs17036314*1/rs12490265*2/rs1801282*1	0.38952	0.05224	0.0495
rs17036314*1/rs4684847*1/rs1801282*1	0.38808	0.05637	0.058
rs17036314*2/rs2938392*1/rs1175542*2	0.28722	0.05607	0.0555
rs12490265*2 /rs1801282*1/ rs2938392*2	0.42052	0.05121	0.0512
rs12490265*2 /rs1801282*1/ rs1175542*1	0.42607	0.05451	0.0558
rs17036314*1/rs12490265*2/rs4684847*1/rs1801282*1	0.38607	0.03466	0.034
rs17036314*2/rs4684847*1/rs2938392*1/rs1175542*2	0.2869	0.05644	0.0564
rs12490265*2/rs4684847*1/rs1801282*1/ rs2938392*2	0.42129	0.0388	0.0387
rs12490265*2/rs4684847*1/rs1801282*1/ rs1175542*1	0.42648	0.04124	0.043

Furthermore, we examined the association between haplotypes built from 7 SNPs and BMI using a global score statistic. While the permutation based global p-value was 0.544 (degree of freedom=11), one individual haplotype gave a p-value of 0.086 (permutation based) in all samples for a haplotype frequency of 0.279. Interestingly, when the analyses were conducted in males only, the permutation based global p-value was 0.055, and one relatively frequent haplotype (frequency =0.28) gave a significant signal of 0.013.

3. Discussion

To summarize, we genotyped the 7 selected SNPs of the PPAR_ transcript variant 3 (including pro12Ala) in a group of unrelated 717 males and 493 females (ages ranging from 11-24), among which 3 SNPs are located in the 5' untranslated region. These 7 SNPs were selected by the tagging algorithm implemented in haploview to cover the whole gene and all SNPs conformed to Hardy-Weinberg equilibrium. We tested each of the 7 SNPs individually for association with body mass index (BMI). Although no association between Pro12Ala and BMI was observed, two SNPs rs2938392 and rs1175542 revealed significant associations with BMI (p-values=0.008 and 0.029, respectively). It is worth noting that the SNP rs2938392 is roughly 41.5 Kb away from Pro12Ala (rs1801282), a polymorphism known to be associated with BMI and diabetes type II. Furthermore, when all 7 SNPs were analyzed together, the permutation based global p-value was 0.544, and one individual haplotype (haplotype frequency =0.279) gave a significant signal of 0.086 (permutation based). Moreover, when the analyses were conducted in males only, the permutation based global p-value was 0.055, and one relatively frequent haplotype (frequency =0.28) gave a significant signal of 0.013.
Several speculations may explain our findings.

1. There is no association between Pro12Ala polymorphism and BMI in the 1,210 school students from Morelos, Mexico. The positive signals we detected are false positives, which may be due to the high-level admixture structure in the Mexican population.
2. There may not be a direct association between Pro12Ala and BMI, however, SNPs which are in high LD with Pro12Ala are associated with BMI in Mexican population, but this hypothesis will need to be further investigated.
3. Environmental factors may play a role to increase the genetic effect and the lack of data on dietary factors and physical activities in this data set may have limited the power to detect an association.

To summarize, our results indicate that SNPs in high linkage disequilibrium with Pro12Ala are associated with BMI in 1,210 students in Morelos, Mexico. However, due to limited access to the epidemiological factors such as dietary factors and physical activities, the results we reported here need to be interpreted with caution. Future study will explore causal associations between other genetic and non-genetic risk factors and obesity in this population. Further studies will consider increased sample sizes, and we will genotype 40 random SNPs to assess the level of population admixture this population.

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