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Amerindians show no association of *PPAR-γ2* gene Ala12 allele and obesity: an "unthrifty" variant population genetics

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Abstract Obesity is for many scholars the most important starting status that gives rise to Metabolic Syndrome (MS) and Type 2 Diabetes (T2D). In the present paper, a genetically homogeneous Amerindian population, as defined by HLA genes, has been genotyped for one of the MS and T2D predisposing genes: PPAR-γ Ala12 and Pro 12 variants. Ala12 has been negatively associated with obesity, but other authors do not find such an association. Notwithstanding, a meta-analysis that used many subjects clearly demonstrated that PPAR-γ Ala12 bearing ones had a reduced risk for T2D. Our results show that Amerindians do not have association of PPAR-γ2 Ala12 and obesity; the latter was measured by waist circumference values after taken specific Amerindian normal waist parameters. Also, a population genetics study indicates that Pro12 allele was the wild allele, which must have occurred before modern humans left Africa. Ala12 may have appeared in Caucasoids later on, according to our comparisons. Negroids tend to show low or null Ala 12 allele frequencies, while most other populations have a significant frequency, particularly European Caucasoids. This may suggest that appearance of Ala12 allele occurred after populations adapted to an agricultural feeding.

Keywords Amerindians · Diabetes · *HLA* · Hypertension · Metabolic Syndrome · Obesity · Population Genetics · *PPAR-γ* Pro12Ala polymorphism · Thrifty genes

Introduction

The metabolic syndrome (MS) has been recently defined like a polygenic disorder that includes visceral obesity, dyslipidaemia, hyperglycaemia and hypertension [1]. It is strongly related with both type 2 diabetes (T2D) and cardiovascular risk factors [2]. Central obesity seems to be the MS initiating principal factor, although some authors put forward data suggesting that insulin resistance is more important as an starting status [1, 2]. However central obesity is T2D independent [3] and a straightforward detection is necessary (i.e.: waist circumference (WC) measurement) [1] because of its higher prevalence among populations, which at present include not only First World ones, but also Africans, Latin Americans and Pacific Islanders [4].

On the other hand, a set of gene variants have been found to be linked to MS components [5, 6] in close interaction with environment. *PPAR-γ* gene (Peroxisome proliferator-activated receptor γ) Pro12Ala SNP from exon 4 which is in contact with gene promoters/ligands in many tissues. The *PPAR-γ2* isoform is only present in adipose tissue [6]. It is a nuclear hormone receptor which regulates

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transcription of genes involved in glucose metabolism, adipocyte differentiation, angiogenesis and inflammation [6]. Also, *PPAR-γ* is a target of therapeutic drugs: thiazolidinediones [7] which improves insulin sensitivity and may be therapeutical for T2D. The Ala12 (Alanine12, A12) variant (G instead C in DNA position 68777 at *PPAR-γ* gene exon 4) has been associated negatively with obesity [8, 9], and in contrast, other authors have associated it positively with obesity [10, 11]. However, meta-analysis with many studies and a huge number of subjects show that *PPAR-γ* Ala12 bearing individuals have a reduced risk for diabetes compared with Pro12 (Proline12, P12, C instead G in DNA position 68777 at *PPAR-γ* gene exon 4) bearing subjects [12, 13]. Other genetic and non genetic factors, like diet, also influence T2D [2].

Nowadays, it is well established that *PPAR-γ* Ala12 variant is associated with reduced risk of MS or its components at least in Caucasians [12, 13]; however, some studies find that is a risk factor for MS or its components [8, 9, 14, 15], and this may be an ethnographic effect. In other words, different populations may bear different *PPAR-γ* gene frequencies both in healthy and diseased people, possible due to different evolutive pressures in their histories as postulated for "thrifty genes" [16]. *PPAR-γ* Ala12 variant different associations and frequencies in ethnic groups may be a reflection of a particular populations different feeding history.

Genetics of about 95 % obesity cases [17] and other MS components [18] include several genes, which bear some polymorphism. Thus, negative association results may be explainable because a non-homogeneous urban population is generally studied. These populations tend to bear many differences at the different relevant and non-relevant genes when compared with more homogeneous ethnic groups and a risk factor for a particular gene is difficult to statistically pin point.

In general, most studies have been made in urban heterogeneous populations whose genetic heterogeneity make more difficult detecting positive or negative linkage of *PPAR-γ* Pro12Ala to obesity or T2D or any metabolic syndrome component [1]. Thus, our present aims are:

(1) To study weather *PPAR-γ* Ala12 variant is a risk factor for a component of MS, obesity, in Amerindians,
(2) To study the possible association in a population model (Amerindians) [19] which is more homogeneous that Caucasoid or Mestizo urban models [19, 20],
(3) To establish the kind of *PPAR-γ* gene association to obesity in Amerindians in order to use Predictive and Preventive Medicine programs both for immigrants and American Amerindians. It is remarkable that about 8 % Madrid in 2006-2011 years interval region population is Amerindian coming from a very recent immigration [20], and
(4) To compare different populations/ethnic groups *PPAR-γ* Ala12 MS risk variant frequencies

Methods and procedures

Population sample

322 unrelated (193 males and 129 females) Amerindian immigrants to Madrid (Spain), aged 21–65 years, were studied. Samples were collected from volunteer Amerindian immigrant blood donors in The Madrid Regional Blood Center. The Amerindian subjects included in the study were selected through external and visible anthropological parameters, in addition to genetic parameters. These genetic parameters are based on specific *HLA-A, -B, -DRB1, and -DQB1* genes from Amerindians, since *HLA* genes in these ethnic groups are very different from those of the rest of the World, including Spaniards [19]. WC was measured to determine obesity in all subjects as described [1]. Subjects included in the obesity group were those whose WC was equal to or greater than 82.5 cm for women or 88.5 cm for men [21]. Serum HDL-cholesterol was determined by an enzyme colorimetric methodology after blocking with monoclonal antibodies all other serum cholesterol fractions. Total cholesterol and triglycerides were also analyzed by an enzymatic colorimetric study. Analyses were carried out by an automatic machine (AU5430, Beckman Coulter Inc., Fullerton, CA, USA). An epidemiological questionnaire was completed by all the volunteer Amerindian blood donors in the study, which included weather they had family history of insulin dependent diabetics and if they gained weight after leaving their country. Informed consent was obtained from all participants in the study, which was approved by the local ethics committees. Individuals with these two characteristics were not included in this study. Written informed consent was obtained from all volunteers with the principles of the Declaration of Helsinki II. The study had been approved by the local Ethics Committee.

PPAR-γ polymorphism screening

Genotyping was carried out on genomic DNA isolated from human leukocytes by specific kit (QuickGene DNA whole blood kit S. Fujifilm LifeScience, Tokio, Japan). Exon 4 *PPAR-γ* gene was amplified by polymerase chain reaction (PCR), using oligonucleotides Fw (*5′-GCCA ATTCAAGCCCAGTC-3′*) and Rw (*5′GATATGTTTGCAG ACAGTGTGTATCAGTGAAGGAATCGCTTTCCG-3′*) as primers. DNA copies of *PPAR-γ* were subjected to specific electrophoresis on agarosa gel and confirmed the PCR

products with a molecular weight marker (Roche Diagnostics GmbH, Mannheim, Germany). Sequencing was carried out in an automated Applied Biosystems ABI PRISM 3700/ABI PRISM 3730 DNA sequencer (Foster City, CA, USA). Sequence of both strands was determined using Chromas 2.31 software and *PPAR-γ* polymorphism was identified by aligning the sequences using Mega 3.1 software.

Statistical analysis

Genotypic and allelic distribution in obese and non-obese individuals was compared calculating chi-square (χ^2) test. The Hardy–Weinberg equilibrium was computed based on the χ^2 goodness-of-fit test performed with the De Finetti programme (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Student's *t* test was used to compare continuous variables expressed as means and standard deviation. Triglyceride and HDL-cholesterol levels were logtransformed prior to analysis in order to obtain normal distribution. Those variables that despite the transformation did not fit to normal distribution were compared using the nonparametric Mann–Whitney U-test. Binary logistic regression analyses were performed to assess the effect of the Pro12Ala polymorphism on obesity after adjustment for covariates like sex, age, biochemical and anthropometric parameters, assuming a dominant and an additive gene effect (the most plausible *PPAR-γ* Pro12Ala polymorphism, according to the literature), as well as a Ala12 allele effect. Adjusted odds ratios (OR) and their 95 % confidence intervals (CI) were estimated. *PPAR-γ* Ala12 allele frequencies in different populations were compared by a Fisher's exact test. Statistical significance was defined as $p < 0.05$. Analyses were performed using the SPSS/PC statistical program (version 14.0 for Windows; SPSS, Inc., Chicago, IL, USA).

Results

Pro12Ala association in the whole Amerindian population (obese and non-obese) with biochemical and anthropometric parameters (Table 1)

The biochemical and anthropometric data in this study for the whole population were compared to *PPAR-γ* genotype (Table 1). No statistically significant link was found in any of the studied parameters and Pro12Ala genotypes. In addition, there were no significant differences in the same characteristics between the Pro/Pro carriers and 12Ala carriers in the obese population and between these same groups in the non-obese population (data not shown).

The frequencies of Pro/Pro genotype and 12Ala carrier genotypes in the whole population were 79.7 and 20.3 % respectively, while the Ala12 allele frequency was 10.5 %. These genotypic and allelic frequencies were very similar to those found in obese and non-obese populations, when obesity is defined by WC. No statistically significant differences in genotypic and allelic distributions in these two populations (obese and non-obese) were found (Table 2). Genotype distributions of the *PPAR-γ* Pro12Ala did not significantly deviate from the Hardy–Weinberg equilibrium.

No Pro12Ala association in obese and non-obese Amerindians (Table 2)

Obesity was defined according to WC values [1, 21]. No significant association was found between 12Ala carriers and obesity ($p = 0.53$), as well as between Ala12 allele and this pathology ($p = 0.41$). No significant association was found neither, when Amerindian population was analysed by separating subjects by sex.

Table 1 Comparison of biochemical and anthropometric data in Amerindian population according to *PPAR-γ* genotypes and alleles

	PPARG genotype		*PPARG* allele (allelic frequency)	
	Pro/Pro	Pro/Ala + Ala/Ala	Pro (0.895)	Ala (0.105)
Gender (f/m)	90/167	39/26	218/359	40/27
Age	38.58 ± 9.04	36.43 ± 10.07	38.34 ± 9.16	36.26 ± 10.08
WC	88.49 ± 11.30	89.30 ± 12.16	88.53 ± 11.36	89.66 ± 12.26
HDL-cholesterol (mg/dl)	46.41 ± 14.01	47.34 ± 13.48	46.53 ± 13.90	47.19 ± 13.65
Total-cholesterol (mg/dl)	215.64 ± 15.44	213.77 ± 57.49	215.71 ± 56.33	211.34 ± 58.45
Triglyceride (mg/dl)	227.01 ± 162.71	246.25 ± 154.88	229.13 ± 161.65	246.83 ± 154.90
SBP	11.52 ± 1.22	11.35 ± 1.23	11.50 ± 1.22	11.33 ± 1.22
DBP	7.34 ± 1.16	7.23 ± 1.44	7.33 ± 1.19	7.19 ± 1.43

Mean values were compared by unpaired Student's *t* and Mann–Whitney *U* tests

WC waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

Table 2 Comparison of obese Amerindian blood donors with *PPAR-γ* genotype and allele frequencies

	Genotypes (%)		Alleles (%)		*p* value*	
	Pro/Pro	Pro/Ala + Ala/Ala	Pro12	Ala12	Genotype	Allele
Total						
Obese	138 (43.12)	38 (11.88)	312 (48.75)	40 (6.25)	0.53	0.41
Non-obese	117 (36.56)	27 (8.44)	261 (40.78)	27 (4.22)		
Males						
Obese	85 (44.27)	17 (8.85)	186 (48.44)	18 (4.69)	0.18	0.14
Non-obese	81 (42.19)	9 (4.69)	171 (44.53)	9 (2.34)		
Females						
Obese	53 (41.41)	21 (16.41)	126 (49.22)	22 (8.60)	0.55	0.70
Non-obese	36 (28.13)	18 (14.06)	90 (35.16)	18 (7.03)		

*Chi-square test

Table 3 Dominance and recessivity *PPAR-γ* Pro12Ala polymorphism comparisons

	Dominant model (Ala/Ala *vs.* Pro/Pro + Pro/Ala)			Additive model (Ala/Ala *vs.* Pro/Ala) = (Pro/Ala *vs.* Pro/Pro)			Ala allele carriers		
	OR	95 % CI	*p* value	OR	95 % CI	*p* value	OR	95 % CI	*p* value
Obesity by WC	1.09[a]	0.50–2.38	0.82	0.79[a]	0.36–1.72	0.55	0.92[a]	0.42–1.99	0.82

WC waist circumference, *OR* odds ratios, *95 % CI* 95 % confidence interval

[a] Adjusted for gender, age, cholesterol, HDL-cholesterol and triglycerides

Testing modes of susceptibility to obesity inheritance caused by *PPAR-γ* Pro12Ala polymorphism (Table 3)

Logistic regression analyses were used to exclude any effect of other available obesity determinants different than *PPAR-γ* genotype, like gender, age and biochemical parameters (cholesterol, HDL-cholesterol, triglycerides). Results in obesity defined by WC show that *PPAR-γ* Ala12 variant was not independently associated to obesity (Table 3).

PPAR-γ Ala12 gene frequency in World populations (Table 4)

Amerindians immigrates to Spain may be added for the first time in the present work to the list of distinct World populations regarding to *PPAR-γ* Ala12 allele frequencies. It is observed that African Negroid (Berba and African Americans), African Ethiopians and Orientals present the lowest frequencies for Ala12 allele, while there is more diversity of Ala12 frequencies in Caucasoid and Amerindian populations (Russians present the highest Ala12 frequency). Amhara (African Ethiopians) Ala12 allele frequency was compared to all other population frequencies using Fisher exact test and found significantly different from those of European Caucasoid and Amerindians.

Discussion

MS and obesity genetics

Visceral obesity is a key and independent component to develop MS (central obesity, raised blood pressure, raised triglycerides, low HDL-cholesterol and fasting hyperglycaemia) [1, 2]. The morbidity of obesity is very high and is the indirect cause of many deaths world wide. At present, even Third World countries (in Latin America, Middles East, Pacific Islands, and also Africa) are suffering a obesity epidemic which has been named ''Globesity'' [22].

It has been put forward the hypothesis of ''thrifty genes'' that have been fixed in our genome for thousands of years to prevent hunger, cold and calamities which hinder feeding [23]. However, exercise can be done by anybody and it is not imprinted in our genes because of evolution, alas it is a main cause of obesity in many families. Other environmental causes of obesity are not imprinted in human genome either (regular feeding, overfeeding). However, with the exception of the existence of single genes causing directly obesity (less than 5 %) [24], most obese people have a polygenic predisposition, in addition to environmental factors to achieve this disease [25]; the evidence for a genetic contribution to obesity has come from epidemiology models. The cause of

Table 4 Differences in *PPARγ* Ala12 allele frequencies in World populations (references in square brackets)

Ethnicity	Population	*n*	Ala12 frequency (%)	*p* value*
Africans				
African Bantu	Berba [42]	49	0.0	0.166
African American	African ancestry in Southwest USA [43]	106	1.9	0.498
African Ethiopian	Amhara [42]	87	2.9	–
Orientals				
	Japanese [44]	3,413	3.0	0.999
	Singapore Chinese [45]	2,730	3.7	0.836
	Han Chinese [46]	148	4.1	0.614
	Koreans [47]	229	4.1	0.641
Caucasoid				
North African	Tunisians [48]	1,062	4.5	0.438
Middle East	Qataris [49]	850	6.0	0.159
	Sikhs [50]	789	12.7†	<0.0001
European	Italians [51]	670	7.0	0.034
	Spanish [52]	462	9.3	0.006
	French [14]	1,711	12.0†	<0.0001
	Danish [53]	2,245	14.0†	<0.0001
	Finnish [37]	1,306	14.5†	<0.0001
	Russians [15]	1,185	20.5†	<0.0001
Amerindians				
	Pima [54]	985	7.8	0.015
	Mexican Mestizo [55]	131	10.0	0.004
	Amerindians immigrants (this study)	320	10.5	0.001
	Mexican Texas [56]	921	12.2†	<0.0001
	Mazahuas [55]	66	16.0†	<0.0001
	Mayans [55]	51	16.0†	<0.0001

Amhara frequencies are confronted with those of all other populations

Pro12 allele frequencies are complementary up to 100 % in all populations

† Comparisons of Ala12 frequencies in Amhara (Ethiopian) population with all other population frequencies. Only population marked like † showed significant different frequencies from Ethiopians

*Fisher's exact test (two-sided) between Amhara Ala12 allele frequency and all other population Ala12 frequencies

-All population frequencies have been compared with all other population frequencies, but only Ethiopian comparisons are shown. Only significant results are depicted

''Globesity'' [26] at present is mostly caused by conducts that favour the predisposing alleles at many genes to work.

It is established that *PPAR-γ* Ala12 variant is an "unthrifty" gene [27], i.e.: it is a protective factor against T2D [12, 13], which is generally associated to obesity and other MS components [2]. Obesity has been postulated to occur independently but may initiate other MS symptomatology [1, 2].

Although this gene variant has been shown in a high number of individuals to protect against MS and associated diseases, it has not been tested in just obese individuals (waist circumference) for a protection to obesity [12, 13].

We have studied direct relationship of this *PPAR-γ* Ala12 gene with obesity in a relatively homogeneous population: Amerindians. This is important because studies in urban heterogeneous unrelated populations may mask positive results of linkage because of a comparatively low *PPAR-γ* protection/predisposition among other genes and the genetic heterogeneity of individuals tested [28–30]. Thus, testing individuals with a more similar genetic background may be useful, as in our case, i.e.: Amerindians monitorized by *HLA* genetics [19, 31] for homogeneity. In addition, different ethnic groups may have different susceptibility gene/alleles, like in other diseases, i.e.: ankilosing spondylites [32].

Amerindians bear a high degree of genetic homogeneity and are different in anthropological and immune genes to the rest of the World [19, 20]. Our sample comes mainly from Andean North-Central highlands [20]. We have not found the expected negative association of Amerindian obese patients with *PPAR-γ* Ala12 allele [12, 13]; this analysis has been carried out by using any of the possible models of inheritance (dominant, additive or recessive, see table 3). Our results may be explained: (1) A sampling error which may be overcome by analyses of much higher Amerindian numbers, (2) Amerindians do not have this polymorphism predisposition. However a higher number of individuals should be analyzed.

PPAR-γ function and genetic polymorphism

Peroxisome proliferator-activated receptor gamma (*PPAR-γ*, NR1C3) belongs to a nuclear receptor superfamily of transcription factor. It regulates adipocyte differentiation and fatty acid uptake and storage [33–35]. In addition, it also may be a signal affecting inflammation, bone morphogenesis, endothelial function, cancer, longevity, atherosclerosis, and even more metabolic human process [36].

For simplicity, we are going only to refer to the Meta-analyses that have shown that *PPAR-γ* Ala12 isoform, present almost exclusively in adipose tissue, protect against MS and T2D [36]. However, we cannot discard that other nearly genetic markers in linkage disequilibrium may be responsible of this effect [36].

Whether pre-existing obesity makes a different *PPAR-γ* Ala12 function in adipogenesis is not well established [37–39] and makes *PPAR-γ* genetic variants effect on MS and obesity very difficult to assess. Then, we only accept solid and extensive publications [12, 13] that demonstrate that *PPAR-γ* Ala12 prevents T2D, MS, obesity and their cardiovascular complications.

PPAR-γ Ala12 MS risk allele in different World populations

We have demonstrated that Amerindians do not have *PPAR-γ* Ala12 as a preventive factor of obesity as measured by WC (Tables 1–3). This finding may be due to a true effect since variant Ala 12 have been show to be an anti-MS factor, at least in Caucasoids. Obesity may be independent and be installed before other MS symptoms, i.e.: T2D [2, 3] and obesity itself (WC) had not previously tested against Pro12Ala variants, and never n Amerindians. Thus our results must be interpreted in this novel context.

The Pro12 allele seems to be the wild allele [27] which must have occurred before modern humans left Africa [27]. Ala 12 allele must have occurred in Caucasoids later on. This allele may induce a slight impairment of transcriptional activation due to decreased DNA-binding affinity [37, 40]. This would result on *PPAR-γ* less activity in promoting adipogenesis, obesity MS and its cardiovascular pathological complications which are nowadays the principal cause of mortality in the First World.

Conversion from hunter-gathering to agricultural economy in "out of Africa" modern humans may have change Pro12 wild allele to harmful, since regular feeding may have driven *PPAR-γ* Pro12 allele to accumulate an energy or fat tissue excess. Subsequent obesity would have led to MS and cardiovascular disease. From the point of view of Evolutionary Medicine another variant (Ala12) with a neutral or slightly *PPAR-γ* dysfunctional effect may have been forced to spread throughout agricultural world due to evolutionary fitness forces. A similar case (but in the opposite direction) may have occurred with the PC-1 Gln121 allele [31].

Table 4 shows that a typical African Negroid population (Berba, African Bantu) shows no Ala12 allele. African American population shows a small Ala12 frequency probably due to admixture and also African Ethiopian population. The latter case may be due to introgression of populations after the first humans left Africa [41].

All other typical Far East Asian populations and North African Caucasoids (Tunisians) show also a Ala12 low frequency. This may reflect: (1) Founder effect, since this mutation may have occurred in out-of-Africa Caucasoids and/or (2) Adaptation to regular agricultural feeding (and Ala12 mutation) occurred later in these populations. Other Caucasoid populations (including North Indian Sikhs) have a high Ala12 mutation frequency. These groups them probably in the original ethnicity in which Ala12 mutation arised. It is interesting that Sikhs go together with Caucasoids in these particular high mutation frequencies and it is a strong evidence for their Caucasoid origin, even in admixture occurred later with Southern Indians. Amerindians, including our sample, show a similar frequency of Ala12 "unthrifty" gene to Caucasoids. This is better explained because of founder effect concordant with that agriculture also started in America about the same times that Mediterranean Eurasia, probably in Oaxaca Valleys (Mexico).

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