

No Evidence for an Association Between Genetic Polymorphisms of β_2 - and β_3 -Adrenergic Receptor Genes With Body Mass Index in Aymara Natives From Chile

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OBJECTIVE: We assessed the association between Gln27Glu and Trp64Arg genetic polymorphisms of the β_2 (ADRB2) and β_3 (ADRB3) adrenergic receptor genes with body mass index and other cardiovascular risk factors.

METHODS: In a cross-sectional study, adult Aymara subjects ($n = 152$) living in the Andean regions of northern Chile were characterized with respect to their ADRB2 and ADRB3 genotypes, body mass index, plasma leptin and insulin levels, fasting glucose concentration, blood pressure, and plasma lipid profile.

RESULTS: The frequency of the Glu27 allele of the ADRB2 gene was estimated to be 0.04, and the allele frequency of the Arg64 variant of the ADRB3 gene was estimated as 0.13. No associations were found between the Trp64Arg polymorphism of the ADRB3 gene and body mass index or other cardiovascular risk factors. The small number of subjects with the allele encoding Glu27 in the ADRB2 gene seriously limited the analysis of the association between genotype and phenotype with the use of this polymorphism, although no clear associations were found.

CONCLUSION: We found insufficient evidence to support an association between polymorphisms Gln27Glu and Trp64Arg of the ADRB2 and ADRB3 genes, respectively, with body mass index and other cardiovascular risks in the rural Aymara population from Chile. *Nutrition* 2002;18:255–258. ©Elsevier Science Inc. 2002

KEY WORDS: body mass index, adrenergic receptor genes, polymorphism, genetics

INTRODUCTION

Researchers have become interested in genes encoding β_2 and β_3 adrenergic receptors as candidate loci of obesity and insulin resistance in humans because of their effects on the regulation of lipolysis and the stimulation of thermogenesis.^{1,2} Further, genetic polymorphisms Gln27Glu in the β_2 adrenergic receptor (ADRB2) gene and Trp64Arg in the β_3 adrenergic receptor (ADRB3) gene have been associated with body mass index (BMI).^{3,4} However, epidemiologic studies have shown discordant results regarding their contributions to the predisposition to overweight or obesity in different populations, with a possible sex-specific effect of the Gln27Glu polymorphism.^{5–9}

The Aymara rural population targeted in this study lives on the western slopes of the Andes in northern Chile, near the borders with Bolivia and Peru. This population preserves ethnically and

culturally distinctive characteristics that are very different from those of the population of European origin living in Chile.¹⁰ Moreover, the Aymara rural population has partly moved from traditional ways of living to the modern lifestyles characteristic of industrialized countries.¹¹ The low prevalence of type 2 diabetes despite relatively high average BMIs and high physical activity levels are remarkable features of this population.¹²

To our knowledge, there are no studies that have evaluated the association between such genetic polymorphisms with BMI or other cardiovascular risk factors in aboriginal populations of South America. Therefore, we assessed the association between Gln27Glu and Trp64Arg genetic polymorphisms of the ADRB2 and ADRB3 genes with BMI, hypertension, and other metabolic complications of obesity in the Aymara population by means of a cross-sectional study.

MATERIALS AND METHODS

Subjects

The target population in this cross-sectional study comprised Aymara adults older than 20 y living in rural conditions at high altitudes (>2000 m) in northern Chile (region of Tarapacá). The recruitment of participants was performed in the context of differ-

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ent health examinations that are carried out periodically and regularly. This study was approved by the Ethics Committee of the University of Chile.

During 1997, apparently healthy subjects older than 20 y were surveyed in the study area ($n = 245$) and blood samples were obtained with informed consent. The percentage of participants with blood type O in the ABO system was 94.8%, which indicates a low admixture with white populations.¹³ Coincidentally, we observed that the subjects with blood type A or B were not permanent residents, and they frequently have European surnames and anthropometric traits of Caucasoid subjects. For this reason, we included only those individual with blood type O and two Aymara surnames, thereby ensuring that the subjects were truly of Aymara origin (Aymara surnames are usually recognizably different from Spanish surnames). Consequently, the sample size was reduced to 152 subjects (60 men and 92 women). Biochemical measurements were determined in blood samples collected between 7:00 and 10:30 AM after an overnight fast of 12 to 14 h. The participants were characterized with the use of standard laboratory automated techniques for fasting plasma glucose concentration, plasma insulin levels, plasma triacylglycerols levels, total plasma cholesterol concentration, and high-density lipoprotein cholesterol levels.¹⁴ Height and weight were determined with the subjects barefoot and lightly clothed. BMI was used as a crude measure of overall adiposity. With the subjects sitting, two measurements of systolic and diastolic blood pressures were recorded with a calibrated digital sphygmomanometer.

Determination of ADRB2 and ADRB3 Genotypes

Polymerase chain reaction (PCR) was performed to amplify specific sequences of the ADRB2 and ADRB3 loci. For both genes, the PCR assay was carried out in a final volume of 30 μ L containing 150 to 250 ng of genomic DNA, 0.5 μ M of each primer, 200 μ M of each dNTP, 1.5 mM of $MgCl_2$, and 1 U of Taq polymerase. All the reagents necessary for this technique, including a 10 \times PCR buffer, were supplied by Bioline (London, UK). The PCR was performed in a Perkin-Elmer GeneAmp 2400 thermocycler (Perkin-Elmer, Oak Brook, IL, USA).

For the ADRB2 gene, a 310–base pair DNA fragment was amplified by using the primers 5'-CCGCCGTGGGTCCGCC-3' (forward primer) and 5'-CCATGACCAGATCAGCAC-3' (reverse primer). PCR was initiated with a denaturation at 94°C for 5 min, followed by 30 cycles (denaturation at 94°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 30 s), with a final extension at 72°C for 7 min. A total of 10 μ L of the PCR product was incubated at 37°C overnight with 2 U of Ital (Roche Molecular Biochemicals, Mannheim, Germany). After incubation with the restriction enzyme, homozygous genotypes for the Glu27 allele variant produced fragments of 171, 84, and 55 base pairs. Homozygous genotypes for the Glu27 allele variant produced fragments of 226 and 84 base pairs.

A 210–base pair DNA fragment of the ADRB3 gene was amplified by using the primers 5'-CGCCCAATACCGCCAACAC-3' (forward primer) and 5'-CCACCAGGAGTCCCATCACC-3' (reverse primer). After heating the PCR mixture at 94°C for 5 min, 35 cycles (denaturation at 94°C for 30 s, annealing at 66°C for 30 s, and extension at 72°C for 30 s) were performed, with a final extension at 72°C for 10 min. An aliquot of 10 μ L of the amplified product was digested at 60°C overnight with 2 U of BstNI (New England Biolabs, Beverly, MA, USA). Homozygous genotypes for the Trp64 variant produced fragments of 97, 61, 31, 15, and 6 base pairs. Homozygous genotypes for the Arg64 allele produced fragments of 158, 31, 15, and 6 base pairs. Restriction fragments from the digestion of ADRB2 and ADRB3 genes were electrophoresed through 2.5% agarose gel and stained with ethidium bromide.

Statistical Methods

Descriptive summary statistics for continuous variables are expressed as mean \pm standard deviation. Differences in BMI and other variables by genotype in ADRB2 or ADRB3 genes were initially assessed with Student's *t* test followed by analysis of covariance.¹⁵ Before the inferential analysis, a logarithmic transformation in leptin concentrations was performed to normalize the distribution of the data. Allele frequencies were estimated and concordance with Hardy–Weinberg proportions were assessed through an exact method.¹⁶ All statistical analyses were done with STATA 6.0 (Stata Statistical Software, College Station, TX).

RESULTS

The frequency of the Glu27 allele in the ADRB2 gene was estimated to be 0.04 (139 homozygous genotypes Glu27/Glu27, 9 heterozygous genotypes, and 1 homozygous genotype Glu27/Glu27). The allele frequency of the Arg64 variant in the ADRB3 gene was estimated as 0.13 (114 homozygous genotype Trp64/Trp64, 37 heterozygous genotypes, and 1 homozygous genotype Arg64/Arg64). Both genes showed genotypic frequencies in concordance with Hardy–Weinberg expectations ($P = 0.17$ for the ADRB2 gene and $P = 0.47$ for the ADRB3 gene).

Table I shows means and standard deviations of continuous variables such as BMI and systolic and diastolic blood pressures and metabolic indicators (plasma fasting glucose, total cholesterol, high-density lipoprotein cholesterol, leptin and insulin concentrations) in relation with the genetic polymorphisms of the ADRB2 and ADRB3 genes. In an initial crude analysis, only the association between the Trp64Arg polymorphism in the ADRB3 gene with diastolic blood pressure achieved statistical significance ($P = 0.01$). After analysis of covariance with sex as the covariate, there was no association between the Glu27Glu and Trp64Arg genetic polymorphisms of the ADRB2 and ADRB3 genes and any of the variables examined in this study except for diastolic blood pressure. The Arg64 carriers on average were 10 y older than the Trp64 homozygotes, and this may be related to the blood pressures in both groups. Therefore, the subsequent adjustment by age and sex led to a non-significant P value of 0.06 for the association between Trp64Arg genetic polymorphism and diastolic blood pressure.

DISCUSSION

The estimated frequency of the Glu27 allele of the ADRB2 gene in the Aymara subjects ($f \approx 4\%$) is roughly within the same range as that estimated for the Quechua population from Peru ($f \approx 0\%$), North American Na-Dene natives ($f \approx 9\%$), and the Japanese ($f \approx 7\%$).^{17,18} Interestingly, allele frequencies in such Amerindian and Asian populations are notably lower compared with European populations from France ($f \approx 40\%$), Spain ($f \approx 37\%$), or North American whites of European ancestry ($f \approx 52\%$).^{8,17,19} With respect to the ADRB3 gene, the prevalence of the allele Arg64 in Aymara subjects was lower than in Pima Indians from Arizona²⁰ and slightly lower than in Japanese individuals. However, the frequency of this polymorphism in Aymaras is still higher than those of most of European populations.⁵ It is important to note that the general Chilean population is a “melting pot” of populations from different groups including Europeans (predominantly from Spain) and Amerindians (mainly Mapuche and Aymara natives).²¹

We found no evidence of an association between the genetic polymorphism of the ADRB2 gene and any of the variables considered in this study. The small number of subjects with the allele encoding Glu27 of the ADRB2 gene seriously limited our assessment of the association between genotype and phenotype when using this polymorphism. As a consequence, it was not possible to assess the effect of sex on the association between

TABLE I.

	MEANS AND STANDARD DEVIATIONS FOR BODY MASS INDEX AND OTHER RELATED VARIABLES IN RELATION WITH THE GENOTYPES OF THE β_2 AND β_3 ADRENERGIC RECEPTOR GENES IN AYMARAS NATIVES FROM CHILE			
	β_3 adrenergic receptor (<i>n</i> = 152)		β_2 adrenergic receptor (<i>n</i> = 149)	
	Trp/Trp (<i>n</i> = 114)	Trp/Arg or Arg/Arg (<i>n</i> = 38)	Gln/Gln (<i>n</i> = 139)	Gln/Glu or Glu/Glu (<i>n</i> = 10)
Age (y)	44.1 ± 18.1	54.0 ± 18.1	45.6 ± 18.8	48.5 ± 16.8
Male/female	46/68	14/24	53/86	3/7
Body mass index (kg/m ²)	25.9 ± 4.3	25.5 ± 4.4	25.5 ± 4.3	26.8 ± 4.4
Fasting plasma glucose (mg/dL)	76.3 ± 28.8	66.8 ± 15.6	75.3 ± 26.7	68.0 ± 22.2
Plasma insulin (μ U/mL)	12.9 ± 15.2	11.9 ± 20.7	12.0 ± 15.1	20.4 ± 30.3
Plasma leptin (μ U/mL)	9.8 ± 10.2	14.6 ± 25.6	9.8 ± 14.4	17.1 ± 21.9
Total plasma cholesterol (mg/dL)	193.6 ± 48.6	196.0 ± 49.1	194.6 ± 48.9	203.7 ± 41.6
Plasma HDL cholesterol (mg/dL)	47.6 ± 20.2	41.6 ± 13.5	46.8 ± 19.1	44.5 ± 13.0
Plasma triacylglycerols (mg/dL)	149.8 ± 82.7	137.0 ± 83.5	138.6 ± 73.3	198.5 ± 127.7
Diastolic blood pressure (mm Hg)	71.5 ± 10.3	76.5 ± 11.8	72.3 ± 11.4	72.9 ± 10.9
Systolic blood pressure (mm Hg)	121.7 ± 17.7	127.2 ± 19.2	121.9 ± 19.2	128 ± 18.9

HDL, high-density lipoprotein

Gln27Glu polymorphism and BMI reported by some investigators.⁸ In a Japanese study,¹⁸ the frequency of the Glu27 allele was significantly higher in obese than in non-obese in male and female subjects. In a French study, where the frequency of the Glu27 allele was much higher than in Aymara or Japanese subjects; the Gln27 allele was associated with higher BMI in men, with no association in women.⁸ Moderate associations between Gln27Glu polymorphism and obesity have been detected in some studies^{4,8} but not in others.^{7,9,22} Therefore, the impact of Gln27Glu polymorphism on the frequencies of overweight and obesity in the Aymaras would depend on the veracity of such an association and, hence, on which allele is associated with higher BMIs. Even assuming that the Glu27 allele truly behaves as a risk factor for obesity in the Aymara population (as in the Japanese population), the low prevalence of this allele is unlikely to have a relevant effect on the occurrence of obesity or obesity-related pathologies. On the contrary, if the Gln27 allele is associated with higher BMI figures, its high frequency in the Aymaras might explain the high prevalence of obesity in this population. It is interesting to note that relative sitting heights (sitting height divided by total height) in Aymara subjects are different than those in subjects of European ancestry²³ and that these differences in body proportions may affect the validity of BMI cutoff points for defining obesity. Further research is specifically needed to refine this issue because it may partly explain the relatively high prevalence of Aymara subjects with BMIs above 30.²⁴ However, the higher means of BMI calculated in this study compared with previous surveys²⁵ may indicate the beginning of a process of epidemiologic transition characterized by changes in dietary habits and physical activity patterns.²⁶

The Aymara population is characterized by a low prevalence of type 2 diabetes, a relatively high average BMI, and high physical activity levels.^{11,12} Most Aymaras living in the Andean regions in northern Chile depend on agriculture for at least part of their subsistence; animal husbandry the secondary source. In this isolated region, the relative uniformity in terms of exposure to environmental risk factors (type of diet or lifestyles) would reduce the effect of confounding factors that could distort our statistical estimates of the association. In addition, the homogeneity of our sample from an ethnic point of view may help to avoid false-positive associations between genotype and phenotype due to population stratification by ethnicity.²⁷ As a drawback of our study, we have to note that selection bias could have affected the

validity of our sample in relation to whether it can be considered representative of our target population. This type of systematic bias might be a consequence of the voluntary participation of subjects in this study. However, it is also important to note that the present survey was conducted as part of a periodic medical survey that regularly follows a large proportion of the Aymara community. The sampling process was carried out in small villages (ranging from 2 to 1203 inhabitants of all ages) with very limited access to health examinations. As a consequence, a large proportion of the population was a priori interested in receiving free medical attention, regardless of their health status, and this circumstance may have reduced the negative impact of such selection bias.

BMI did not correlate significantly with the genetic Trp64Arg polymorphism of the ADRB3 gene in Aymara natives. Controversial results regarding the effect of this polymorphism on BMI have been reported in the past, including a possible interaction between age and obesity.^{5,28-29} Likewise, no association was found between this genetic variant and the other metabolic complications of obesity measured in this study (Table I). Nevertheless, a nearly significant association was detected between the Trp64Arg genetic polymorphism and systolic blood pressure after controlling for sex and age in the statistical analysis. Although some studies have suggested an association between this polymorphism and hypertension,³⁰ it is interesting to note the particular situation of our Aymara population in which a gradient in the blood pressure by altitude has been described. According to this gradient, Aymara inhabitants living 4000 m above sea level would have a decreased average of systolic blood pressure than people living lower altitudes.³¹ Therefore, we should consider the hypothesis that differences in blood pressure by ADRB3 genotype may be attributable to differences according to altitude. Unfortunately, it was difficult to evaluate the role of altitude in our study because the inferential analysis was severely limited by the sampling variation caused by small sample sizes resulting from the stratification by altitude, sex, age, and ADRB3 genotype. As a consequence, we could not establish a solid strategy to assess the relation between the Trp64Arg polymorphism of the ADRB3 gene and hypertension in the Aymara population.

In conclusion, the present study does not provide sufficient evidence to support the association between the genetic polymorphisms Gln27Glu and Trp64Arg of the ADRB2 and ADRB3 genes

with BMI and other cardiovascular risk in the rural Chilean Aymara population.

REFERENCES

- Arner P, Hoffstedt J. Adrenoreceptor genes in human obesity. *J Intern Med* 1999;245:667
- Macho-Azcarate T, Marti del Moral A, Martinez-Hernandez JA. Estudios genéticos de la obesidad en humanos. *Med Clin* 2000;115:103
- Clement K, Vaisse C, Manning BS, et al. Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 1995;333:352
- Large V, Hellstrom L, Reynisdottir S, et al. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest* 1997;100:3005
- Allison DB, Heo M, Faith MS, Pietrobelli A. Meta-analysis of the association of the Trp64Arg polymorphism in the beta3 adrenergic receptor with body mass index. *Int J Obes* 1998;22:559
- O'Dell SD, Bolla MK, Miller GJ, et al. W64R mutation in beta-3-adrenergic receptor gene and weight in a large population sample. *Int J Obes* 1998;22:377
- Oberkofler H, Esterbauer H, Hell E, Krempler F, Patsch W. The Gln27Glu polymorphism in the beta2-adrenergic receptor gene is not associated with morbid obesity in Austrian women. *Int J Obes* 2000;24:388
- Meirhaeghe A, Helbecque N, Cotel D, Amouyel P. Impact of polymorphisms of the human beta2-adrenoceptor gene on obesity in a French population. *Int J Obes* 2000;24:382
- Rosmond R, Ukkola O, Chagnon M, Bouchard C, Bjorntorp P. Polymorphisms of the beta2-adrenergic receptor gene (ADRB2) in relation to cardiovascular risk factors in men. *J Intern Med* 2000;248:239
- Rothhammer F, Spielman RS. Anthropometric variation in the Aymara: genetic, geographic, and topographic contributions. *Am J Hum Genet* 1972;24:371
- Santos JL, Pérez-Bravo F, Albala C, Calvillán M, Carrasco E. Leptin levels in Chilean native Aymara subjects. *Ann Hum Biol* 2000;27:271
- Santos JL, Pérez-Bravo F, Carrasco E, Calvillán M, Albala C. Low prevalence of type 2 diabetes despite a high average body mass index in the Aymara natives from Chile. *Nutrition* 2001;17:305
- Valenzuela CY, Acuña MP, Harb Z. Gradiente sociogenético en la población chilena. *Rev Med Chil* 1987;115:295
- Pérez-Bravo F, Albala C, Santos JL, Yáñez M, Carrasco E. Leptin levels distribution and ethnic background in two populations from Chile: Caucasian and Mapuche groups. *Int J Obes* 1998;22:943
- Kleinbaum DG, Kupper LL, Muller RT. *Applied regression analysis and other multivariable methods*. Boston: PWS Kent, 1988
- Cleves M. Hardy-Weinberg equilibrium test and allele frequency estimation. *Stat Tech Bull* 1999;48:34
- Rupert JL, Monsalve MV, Devine DV, Hochachka PW. Beta2-adrenergic receptor allele frequencies in the Quechua, a high altitude native population. *Ann Hum Genet* 2000;64:135
- Ishiyama-Shigemoto S, Yamada K, Yuan X, et al. Association of polymorphisms in the β 2-adrenergic receptor gene with obesity, hypertriglyceridaemia, and diabetes mellitus. *Diabetologia* 1999;42:98
- González-Sánchez JL, Porenza AM, Martínez-Larrad MT, et al. Beta2/beta3 adrenergic receptor genes polymorphism in the general population of Spain according to BMI and degree of glucose tolerance. *Int J Obes* 2000;24:S36
- Walston J, Silver K, Bogardus C, et al. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the beta 3-adrenergic-receptor gene. *N Engl J Med* 1995;10:343
- Cruz-Coke R. Origen y evolución étnica de la población chilena. *Rev Med Chil* 1976;104:365
- Kortner B, Wolf A, Wendt D, Beisiegel U, Evans D. Lack of association between a human beta-2 adrenoceptor gene polymorphism (gln27glu) and morbid obesity. *Int J Obes* 1999;23:1099
- Palomino H, Mueller WH, Schull WJ. Altitude, heredity and body proportions in Northern Chile. *Am J Phys Anthropol* 1979;50:39
- Norgan NG. Population differences in body composition in relation to the body mass index. *Eur J Clin Nutr* 1994;48(suppl 3):S10
- Mueller WH, Murillo F, Palomino H, et al. The Aymara of western Bolivia: growth and development in an hypoxic environment. *Hum Biol* 1980;52:529
- Albala C, Vio F, Kain J, Uauy R. Nutrition transition in Latin America: the case of Chile. *Nutr Rev* 2001;59:170
- Altshuler D, Kruglyak L. Genetic polymorphisms and disease. *N Engl J Med* 1998;338:1626
- Fujisawa T, Ikegami H, Kawaguchi Y, Ogihara T. Meta-analysis of the association of Trp64Arg polymorphism of beta 3-adrenergic receptor gene with body mass index. *J Clin Endocrinol Metab* 1998;83:2441
- Corbalán MS, Marti A, Martínez-González MA, Forga L, Martínez JA. The influence of a β 3-adrenergic polymorphism on the risk of obesity is evident in young adults. *Int J Obes Relat Metab Disord* 2001;25(suppl 2):S45
- Tonolo G, Melis MG, Secchi G, et al. Association of Trp64Arg beta 3-adrenergic-receptor gene polymorphism with essential hypertension in the Sardinian population. *J Hypertens* 1999;17:33
- Makela M, Barton SA, Schull WJ, Weidman W, Rothhammer F. The multinational andean genetic and health program—IV. Altitude and the blood pressure of the Aymara. *J Chron Dis* 1978;31:587