Endocrine Journal 2007, **54** (1), 89–94

LEPR, ADBR3, IRS-1 and 5-HTT Genes Polymorphisms do not Associate with Obesity

HATİCE MERGEN, ÇAĞATAY KARAASLAN, MEHMET MERGEN*, ERGİ DENİZ ÖZSOY** AND METİN ÖZATA***

Department of Molecular Biology, Faculty of Science, Hacettepe University, 06532, Ankara, Turkey
**Ankara Military Hospital, Department of Internal Medicine, 06610, Diskapi, Ankara, Turkey*
***Department of Biology, Faculty of Science, Hacettepe University, 06532, Ankara, Turkey*
****GATA Haydarpaşa Training Hospital, Department of Endocrinology and Metabolism, 34660, Acibadem, Istanbul, Turkey*

Abstract. Obesity is a growing problem and is associated with numerous medical conditions. In several genes coding for molecules involved in the regulation of body weight (fat mass) and thermogenesis, polymorphisms have been reported which possibly modify human obesity risk. The aim of this study was to determine the incidence of the following polymorphisms in the following genes in 262 obese (BMI≥30) and 138 control (BMI≤25) subjects: leptin receptor (*LEPR*)-*Gln223Arg*, B_3-adrenergic receptor (B_3-AR)-Trp64Arg, serotonin transporter (*5-HTT*)-a 44-base pair insertion/deletion functional polymorphism in the 5-HTTLPR and insulin receptor substrate-1 (*IRS-1*)-*Gly972Arg*. Our hypothesis was that these polymorphisms would occur more frequently in the obese population. The polymorphisms were determined by polymerase chain reaction (PCR) and restriction genotyping in study population. In our results, no strong associations were observed between BMI status and these polymorphisms. Weak, though significant, association coefficients obtained with HTT and LEPR loci indicate that the genotype numbers at these loci may depend on BMI status to some extent.

Key words: B_3-adrenergic receptor, Insulin receptor substrate-1, Serotonin transporter, Leptin receptor, Obesity, PCR, Polymorphism, RFLP

(*Endocrine Journal* **54**: 89–94, 2007)

OBESITY is a major health problem worldwide influenced by both genetic and environmental factors and the risk of becoming obese has a strong genetic component [1]. Since single gene defects resulting in obesity are very rare, it is likely that a combination of polymorphisms in one or more candidate genes may contribute to its development. Using this approach, many candidate genes including β3-adrenergic receptor, leptin receptor, serotonin transporter, and insulin receptor substrate-1 gene have been assessed for association with obesity in several studies [2].

The human B_3-AR is expressed specifically in adipose tissues, and activated in brown adipose tissues during the thermogenesis and in white adipose tissues during the lipolysis [3]. Several studies have suggested that a missense *Trp64Arg* mutation in the B_3-adrenergic receptor (*ADRB3*) gene is involved in obesity and insulin resistance [4–7].

Leptin is an adipose-derived cytokine present in the circulation in amounts proportional to fat mass that acts to reduce food intake and increase energy expenditure thereby regulating body weight homeostasis and the weight-regulating effects of leptin are mediated through the binding and activation of the long isoform of its receptor (LEPR-b) in the hypothalamus. *LEPR*, has defined a novel molecular pathway for energy metabolism and regulation of body weight [8]. The *Gln223Arg* polymorphism in the *LEPR* gene has been reported to be associated with obesity phenotypes [9–11].

5-HTT is responsible for the sodium-dependent re-

Received: February 2, 2006
Accepted: September 25, 2006
Correspondence to: Hatice MERGEN, Ph.D., Hacettepe University, Faculty of Science, Department of Molecular Biology, 06532, Ankara, Turkey

uptake of serotonin from the synapse after its release from serotonergic neurons [12] and plays a key role in regulating synapse levels of available serotonin and serotonin turnover [13]. The human *5-HTT* gene has a functional polymorphism in the 5'-flanking promoter region that is described: a 44-base pair (bp) insertion/deletion in the 5-HTT gene-linked polymorphic region (5-HTTLPR; two alleles: "long" [l] and "short" [s]) [14, 15]. The transcriptional activity of the long form allele was more than twice as high as that of the short form allele [16]. As far as we know, four studies have examined the association between 5-HTTLPR and eating disorders (ED) [17–20].

The insulin receptor is part of a transmembrane tyrosine kinase-mediating intracellular signaling process that leads to the biological actions of insulin [21]. *Arg972Gly*, a common variant of *IRS-1* gene are associated with obesity and type 2 diabetes in some populations. In obese adults, Arg972Gly appears to increase insulin resistance in its heterozygous form [22].

Since these findings strongly suggest the existence of an interaction between leptin receptor (LEPR)-*Gln223Arg*, B_3-adrenergic receptor (B_3-AR)-*Trp64Arg*, serotonin transporter (5-HTT)-a 44-base pair insertion/deletion functional polymorphisms and obesity, we compared the prevalence of these changes in obese subjects and a group of lean subjects in Turkish population.

Material and Methods

Subjects

262 obese subjects, (159 female and 103 male) (BMI; 39.0722 ± 0.3586) and 138 control subjects (55 female and 84 male) (BMI; 21.7391 ± 0.1897) were enrolled into the study. The obese subjects were recruited from the outpatient clinic of Department of Endocrinology and Metabolism of Gülhane Military School of Medicine in Turkey. Body mass index (BMI) values were greater than 30 kg/m^2 in obese subjects. All obese subjects had a history of severe obesity before the age of 10 and had at least one other obese family member. None of the obese subjects had diabetes mellitus or impaired glucose tolerance in this study. Control subjects underwent routine physical and laboratory evaluations to ensure that none had obesity, diabetes mellitus, hyperlipidemia, psychiatric, metabolic, hepatic or renal disease. None of the control subjects had a family history of hyperlipidemia or diabetes. The study was approved by the local ethical committee of Gülhane Medical School.

DNA studies

Genomic DNA was extracted from white blood cells using phenol-chloroform extraction. The *Trp64Arg* of *ADRB3* gene, *Gln223Arg* of *LEPR* gene and *Gly972Arg* of *IRS-1* gene polymorphisms were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using BstN I, Msp I and Sma I endonuclease enzymes digestion, respectively, according to the methods described in the literature [4, 6, 23, 24]. The 5-HTTLPR genotype was determined by the method of Lesch *et al.* [25].

Statistical analysis

All the statistical analysis was performed with SPSS 10.0 (SPSS Inc., Chicago, IL, USA) statistical package. Body mass indices of obese and lean subjects were analyzed on genotypic grounds by single classification ANOVA. Since the test chosen (Levene's Test) for the homoscedasticity of the group variances showed significant heterogeneity among the studied groups, a *post hoc* test that does not assume variance equality, Games-Howell, was performed in the analyses [26]. Allelic differences were tested using a χ^2 which uses the weighted variance and weighted average of allele frequencies over the samples compared [27]. In this analysis female and male genotypes were pooled as there were no significant sex differences in obesity on genotypic grounds (not shown).

Results

Mean BMI values of lean and obese samples were estimated. A *t*-test was performed for assessing significancy of the difference between them. Individual BMI values were logarithmically transformed for normality and variance homogeneity because the non-transformed original measurements of the two groups were not distributed normally and had nonhomogenous variances (not shown). The *t*-test was the one appropriate for the unequal sample sizes with a large difference [26]. A *t*-test shows the expected highly significant difference between lean and obese samples with re-

Table 1. Allele frequencies of the 4 loci studied and deviations from Hardy-Weinberg equilibriums expressed as χ^2 values. Allelic frequency differences across lean and obese samples are also shown per loci expressed as χ^2 values obtained by the method of Workman and Niswander (1970).

Locus/genotype	Genotype Number		Allele Frequency		$\chi^{2,a}$		$\chi^{2,b}$
	Lean	Obese	Lean	Obese	Lean	Obese	
5-HTT							
(*SS*)	47	74					
(*SL*)	67	117	*0.588* (*S*)	*0.506* (*S*)	0.01	2.98	5.58*
(*LL*)	23	71	0.412 (L)	0.494 (L)			
LEPR							
(*Gln223Gln*)	65	92					
(*Gln223Arg*)	61	141	*0.692* (*Gln223*)	*0.620* (*Gln223*)	0.19	5.31*	4.19*
(*Arg223Arg*)	12	29	*0.318* (*Arg223*)	*0.380* (*Arg223*)			
ADBR3							
(*Trp64Trp*)	120	226					
(*Trp64Arg*)	18	35	*0.935* (*Trp64*)	*0.929* (*Trp64*)	0.03	0.06	0.62
(*Arg64Arg*)	0	1	*0.065* (*Arg64*)	*0.071* (*Arg64*)			
IRS-1							
(*Gly972Gly*)	122	238					
(*Gly972Arg*)	16	23	*0.942* (*Gly972*)	*0.952* (*Gly972*)	0.14	0.31	1.64
(*Arg972Arg*)	0	1	0.058 (*Arg972*)	0.048 (*Arg972*)			

[a] Deviation from Hardy-Weinberg equilibrium
[b] Allele frequency difference between lean and obese samples
* P<0.05

spect to BMI (t = 14.936, d.f. = 120, P<0.001). Estimated allele frequencies and relevant statistics are shown in Table 1. Deviations from Hardy-Weinberg equilibriums were tested using conventional χ^2 test with categories having expected genotype numbers less than 5 added to second largest category (*i.e.*, *ADBR3* and *IRS*). Only *LEPR* locus of obese sample showed significant deviation from Hardy-Weinberg equilibrium ($\chi^2 = 5.31$, d.f. = 1, P<0.05, Table 1). This significant deviation at *LEPR* is due to higher frequency of the mutant allele (*Arg223* = 0.380) in the obese sample. Allele frequency differences between lean and obese samples were also tested for significancy. The test performed was the one by Workman and Niswander (1970) [27], in which weighted average variance of allele frequencies between any two samples is used to obtain a χ^2 test value. *HTT* and *LEPR* normal allele frequencies seem to be significantly different between lean and obese samples ($\chi^2 = 5.58$ (*HTT*) and $\chi^2 = 4.19$ (*LEPR*), d.f. = 1, P<0.05). This is due to a higher frequency of the mutant allele in the obese sample in both cases (*L* = 0.494 and *Arg223* = 0.380, respectively). As there was no significant allele frequency difference between sexes per loci (not shown) sex-pooled numbers were used in the tests. Whether there was an

Table 2. Association levels of HTT and LEPR genotypes with BMI status of the obese sample as described by an association coefficient, phi (Φ)

Locus	G-value	Association coefficient, Φ
5-HTT	5.90	0.121
LEPR	5.35	0.116

association of genotype numbers at *HTT* and *LEPR* loci with obesity was tested by an R × C test of independence in a 3 (genotypes) × 2 (BMI statuses as lean and obese) design (see Sokal and Rohlf, 1995, p. 738, for details) [26]. G-values obtained by the test and the phi (Φ) coefficients of association for each genotype are shown in Table 2. Although estimated G-values (*i.e.*, associated χ^2 values for d.f. = (3–1) × (2–1) = 2) are not significant, they are quite close to the border of significant deviation (*i.e.*, tabled $\chi^2 = 5.991$, d.f. = 2, P = 0.05). Association coefficients indicate that the genotype numbers at *HTT* and *LEPR* loci may depend on BMI status, though not strongly. To see whether genotypic combinations of alleles may affect obesity level, we performed analyses in the obese group using genotype specific BMI values for all the loci (*i.e. HTT*, *LEPR*, *ADBR3*, and *IRS*). In that respect, one-way

Table 3. Mean BMI differences among genotypes within particular loci. The mean BMI values were estimated only for the obese individuals on respective genotypic bases. One-way ANOVA results are given as F values with their statistical assesments.

Locus	Genotype	n	Mean BMI	±SE	F
5-HTT	*SS*	27	39.92	0.72	
	SL	55	38.47	0.79	0.565 NS
	LL	26	39.30	1.49	
LEPR	*Gln223Gln*	40	39.70	0.99	
	Gln223Arg	63	38.56	0.70	0.395 NS
	Arg223Arg	5	41.60	2.77	
ADBR3	*Trp64Trp*	92	38.54	0.57	
	Trp64Arg	16	42.44	1.75	0.013*
IRS-1	*Gly972Gly*	93	38.85	0.57	
	Gly972Arg	12	41.67	2.44	0.121

n: number of obese individuals
SE: Standard error of the mean
* P<0.05
NS: Not significant

ANOVAs were performed for each loci in which BMI was dependent variable and genotypes for a locus were treated as groups. Table 3 gives the descriptive data and statistical assesments for mean BMI differences among genotypes within particular loci. For the *5-HTT* and *LEPR* loci, mean BMIs among the genotypes were not significantly different. For the *ADBR3* locus, genotype based mean BMIs were estimated only for the *Ttrp64Trp* homozygote and *Trp64Arg* heterozygote as there was only one *Arg64Arg* homozygote individual found in our study. The mean BMI difference between the *Trp64Trp* homozygote and *Trp64Arg* heterozygote was significant (F = 0.013, P<0.05, Table 3). As for the *IRS-1*, only two genotypic categories (*Gly972Gln* and *Gly972Arg*) existed, the difference between respective BMIs being not significant this time (F = 0.121, Table 3). The striking similarity between *ADBR3* and IRS-1 is that when the common allele (*i.e. Trp64* and *Gly972*, respectively) is halved in number in heterozygotes, there seems a dose affect raising the mean BMI values (Mean BMIs of *ADBR3* and *IRS-1* heterozygotes, Table 3). But the small number of heterozygotes available for the each locus and the large standard errors for heterozygote BMIs (Table 3, SE) ensuing make it more likely a sampling effect stemming from the rarity of the mutant alleles in the respective loci (*Arg64* in *ADBR3* and *Arg972* in *IRS-1*, Table 1).

Discussion

In this study we investigated the effects of four common polymorphisms in the *5-HTT*, *LEPR*, *ADBR3* and *IRS-1* genes, namely the *5-HTTLPR*, *Q223R*, *Trp64Arg* and *Gly972Arg* polymorphisms, on BMI in a Turkish obese and lean individuals. Although no association between BMI status and these polymorphism was observed, there was a weak, but significant, association coefficients indicating that the genotype numbers at *HTT* and *LEPR* loci may depend on BMI status, though not strongly (Table 1, 2). Similarly, no significant effect on gender on body mass indices was detected within each genotype or among the different genotypes in this study (data not shown). We also performed analyses in the obese group using genotype specific BMI values for all the loci (*i.e. HTT*, *LEPR*, *ADBR3*, and *IRS*) to see whether genotypic combinations of alleles may affect obesity level. Though, in halving the number of common alleles, there seems a dose affect raising the mean BMI values in two of the loci (*ADBR3* and *IRS-1* heterozygotes, Table 3), small number of heterozygotes available for the each locus and the large standard errors for heterozygote BMIs ensuing make it more likely a sampling effect stemming from the rarity of the mutant alleles in the respective loci.

Overall, these findings are in accordance with recent studies in which no difference has been found in the allelic frequencies of the *LEPR* and *ADBR3* genes polymorphisms between obese and nonobese subjects. For example, linkage analysis in the Quebec Family Study Cohort has indicated no association of the Arg allele with obesity-related phenotypes [28]. Silver *et al.* have shown that *Gln223Arg* polymorphism in the human leptin receptor do not associate with traits related to obesity [29].

Obesity is a major public health concern given the association of this condition with several chronic diseases. Identification of genetic variants that increase a person's susceptibility to the common forms of obesity is a critical problem. Several recent studies have made an attempt in this direction. Studies in Pima Indians [4], French Caucasians [5], Finns [6], Japanese [7] have shown of a modest association of the Arg allele with various anthropometric markers of obesity and diabetes.

Several studies have suggested that a common polymorphism *Gln223Arg* in the *LEPR* gene is associated with obesity phenotypes. For example, this association

was observed in middle-aged Caucasian males [9], postmenopausal Caucasian women [10], a Mediterranean population [11].

As far as we know, four studies have examined the association between 5-HTTLPR and ED. Fumeron *et al.* reported a higher frequency of the S/S genotype of 5-HTTLPR in AN subjects than in normal-weight controls [17], but reports of evidence against these findings [18–20] make the association controversial.

Mutations in the *IRS-1* gene are associated with obesity and type 2 diabetes in some populations. In obese adults, however, *Arg972Gly* appears to increase insulin resistance in its heterozygous form [22].

We know that the confounding factor of the several studies may be due to a small size of study groups and ethnic differences of them. It must be noted, however, our analyses were carried out on randomly chosen samples in terms of ethnic origin, hence no different ethnic compositions in the case and control samples (Turkish population). Though the correlation profile of B3-AR and IRS-1 may be due to relatively small sample size, we think that this situation could also arise from different contributions of different polymorphisms to BMI status. On the other hand, in the obesity panel, the subjects were obese before the age of ten. This might arise the possibility that they may have very strong genetic background, and that the effects of the polymorphisms we examined may have been masked. Those polymorphisms may contribute to milder form of obesity in Turkish population.

In conclusion, our study does not provide evidence for a major role of the *5-HTT*, *LEPR*, *ADBR3* and *IRS-1* genes, namely the *5-HTTLPR*, *Q223R*, *Trp64Arg* and *Gly972Arg* polymorphisms, in predisposition to obesity. As our knowledge of obesity genes advances with new discoveries, further studies of well defined obesity phenotypes and associated gene mutations or polymorphisms in larger samples may be helpful to investigate a more subtle effect of these genes in this serious phenotype. Such studies should also consider possible interactions with other genetic polymorphisms.

Acknowledgements

This work was supported by the State Planning Organization DPT-02K120290-12 and Hacettepe University Research Foundation 0101601006 to H. Mergen, PhD. Part of this work was supported by Servier and GATA Resarch Center to M.Ozata, M.D.

References

1. Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB (1999) Annual deaths attributable to obesity in the United States. *JAMA* 282: 1530–1538.
2. Rosmond R (2003) Association studies of genetic polymorphisms in central obesity: a critical review. *Int J Obes Relat Metab Disord* 27: 1141–1151.
3. Inukai T, Tayama K, Inukai Y, Matsutoma R, Takebayashi K, Aso Y, Takemura Y (2001) Clinical features of a polymorphism of the β_3-adrenergic receptor gene in patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 109: 386–388.
4. Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, Sorkin JD, Roth J, Shuldiner AR (1995) Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic receptor gene. *N Engl J Med* 333: 343–347.
5. Clement K, Vaisse C, Manning BS, *et al.* (1995) Genetic variation in the beta(3)-adrenergic-receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 333: 352–354.
6. Widen E, Lehto M, Kanninen T, Walston J, Schuldiner A, Groop LC (1995) Association of a polymorphism in the β3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 333: 348–351.
7. Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, Watson J, Yoshinaga H, Kosaka K, Yamada N (1995) A mutation in the beta(3)-adrenergic-receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. *Biochem Biophys Res Commun* 215: 555–560.
8. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* 395: 763–770.
9. Chagnon YC, Wilmore JH, Borecki IB, Gagnon J, Perusse L, Chagnon M, Collier GR, Leon AS, Skinner JS, Rao DC, Bouchard C (2000) Associations between the leptin receptor gene and adiposity in middle-aged Caucasian males from the HERITAGE family study. *J Clin Endocrinol Metab* 85: 29–34.
10. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI (2001) A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and

leptin levels in postmenopausal Caucasian women. *Hum Genet* 108: 233–236.

11. Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS (2001) The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab* 86: 4434–4439.
12. Rudnick G, Clark J (1993) From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta* 1144: 249–263.
13. Bellivier F, Szoke A, Henry C, Lacoste J, Bottos C, Nosten-Bertrand M, Hardy P, Rouillon F, Launay JM, Laplanche JL, Leboyer M (2000) Possible association between serotonin transporter gene polymorphism and violent suicidal behavior in mood disorders. *Biol Psychiatry* 48: 319–322.
14. Heils A, Teufel A, Petri S, Seemann M, Bengel D, Balling U, Riederer P, Lesch KP (1995) Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. *J Neural Transm Gen Sect* 102: 247–254.
15. Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. *J Neurochem* 66: 2621–2624.
16. Collier DA, Stober G, Li T, Heils A, Catalano M, Di Bella D, Arranz MJ, Murray RM, Vallada HP, Bengel D, Muller CR, Roberts GW, Smeraldi E, Kirov G, Sham P, Lesch KP (1996) A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. *Mol Psychiatry* 1: 453–460.
17. Fumeron F, Betoulle D, Aubert R, Herbeth B, Siest G, Rigaud D (2001) Association of a functional 5-HT transporter gene polymorphism with anorexia nervosa and food intake. *Mol Psychiatry* 6: 9–10.
18. Hinney A, Barth N, Ziegler A, von Prittwitz S, Hamann A, Hennighausen K, Pirke KM, Heils A, Rosenkranz K, Roth H, Coners H, Mayer H, Herzog W, Siegfried A, Lehmkuhl G, Poustka F, Schmidt MH, Schafer H, Grzeschik KH, Lesch KP, Lentes KU, Remschmidt H, Hebebrand J (1997) Serotonin transporter gene-linked polymorphic region: Allele distributions in relationship to body weight and in anorexia nervosa. *Life Sci* 61: 295–303.
19. Di Bella DD, Catalano M, Cavallini MC, Riboldi C, Bellodi L (2000) Serotonin transporter linked polymorphic region in anorexia nervosa and bulimia nervosa. *Mol Psychiatry* 5: 233–234.
20. Sundaramurthy D, Pieri LF, Gape H, Markham AF, Campbell DA (2000) Analysis of the serotonin transporter gene linked polymorphism (5-HTTLPR) in anorexia nervosa. *Am J Med Genet* 96: 53–55.
21. White MF (1997) The insulin signalling system and the IRS proteins. *Diabetologia* 40: 2–17.
22. Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K, *et al.* (1995) Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. *Lancet* 346 : 397–402.
23. Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y, Nishi S, Thompson DB, Nakao K (1997) Human leptin receptor gene in obese Japanese subjects: evidence against either obesity causing mutations or association of sequence variants with obesity. *Diabetologia* 40: 1204–1210.
24. Baroni MG, D'Andrea MP, Montali A, Pannitteri G, Barilla F, Campagna F, Mazzei E, Lovari S, Seccareccia F, Campa PP, Ricci G, Pozzilli P, Urbinati G, Arca M (1999) A common mutation of the insulin receptor substrate-1 gene is a risk factor for coronary artery disease. *Arterioscler Thromb Vasc Biol* 19: 2975–2980.
25. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 29; 274: 1527–1531.
26. Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. 3rd. Edition. W.H. Freeman and Co., New York, p. 887.
27. Workman PL, Niswander JD (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am J Hum Genet* 22: 24–49.
28. Gagnon J, Mauriege P, Roy S, Sjostrome D, Chagnon YC, Dionne FT, Oppert JM, Perrusse L, Sjostrom L, Bouchard C (1996) The Trp64Arg mutation of the beta 3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec family study and Swedish obese subjects cohorts. *J Clin Invest* 98: 2086–2093.
29. Silver K, Walston J, Chung WK, Yao F, Parikh VV, Andersen R, Cheskin LJ, Elahi D, Muller D, Leibel RL, Shuldiner AR (1997) The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. *Diabetes* 46: 1898–1900.