β-3AR W64R Polymorphism and 30-Minute Post-Challenge Plasma Glucose Levels in Obese Children

Hasibe Verdi¹, Sibel Tulgar Kı nik², Yaparak Yılmaz Yalçın¹, Nursel Muratoğlu Şahin², Ayşe Canan Yazıcı³, F. Belgin Ataç¹

¹Başkent University Faculty of Medicine, Department of Medical Biology, Ankara, Turkey
²Başkent University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey
³Başkent University Faculty of Medicine, Department of Biostatistics, Ankara, Turkey

Introduction

Obesity is a result of an imbalance between nutrient intake and energy expenditure. Increased positive energy balance causes fat storage. With increasing epidemics of obesity all over the world, recent research focused on both genetic and environmental factors affecting the energy balance in children and adolescents (1,2,3).

The sympathetic nervous system plays an important role in the regulation of energy expenditure. Catecholamines are powerful regulators of lipolysis and act via β-adrenoceptors (β-ARs). Thus, β-ARs play important roles in energy expenditure and body weight. Base variation in the β-3AR causes the substitution of the coding sequences from tryptophan (W) into arginine (R) in 64th position, a change that influences the affinity of the receptor to norepinephrine. Masuo et al (4) have reported close relationships between β-2 and β-3AR polymorphisms accompanying elevated sympathetic nervous activity, hypertension, obesity and insulin resistance in a longitudinal study. β-3ARs are located mainly in the adipose tissue and are involved in the regulation of lipolysis and thermogenesis by catecholamines, as well as in the development of obesity (5).

In a number of previous studies, β-3AR genotype (mainly W64R) was associated with obesity and related disorders such as hypertension, increased waist-to-hip ratio, cardiovascular disease, dyslipidemia, insulin resistance and metabolic syndrome in adults (6,7,8,9,10,11,12,13). However, these findings have not been confirmed in other studies (14,15,16).

In children, reported data are limited and discordant. In some studies with children, R64 allele was not found to be related to
obesity (17,18,19). In contrast, in other studies, W64R variant was found to be associated with obesity (20,21,22).

In a recent study with obese children, W64R polymorphism was found to be significantly associated with metabolic syndrome components such as increased visceral fat, dyslipidemia, higher blood pressure (23). Although β-3AR genotype was found to be related with insulin resistance (IR), there is scarce data on results of oral glucose tolerance test (OGTT).

In this study, we aimed to investigate W64R polymorphism of the β-3AR gene in obese children and also the relationship between genotype and obesity-related metabolic disorders. The association between β-3AR genotype and glucose-insulin levels during OGTT was also investigated.

Methods

Two hundred fifty-one unrelated children and adolescents were enrolled in the study. Of these cases, 130 were obese and 121 constituted the healthy control group. The DNA studies were conducted on peripheral blood for β-3AR W64R genotyping of the children in the obese and control groups. In the obese group, IR and dyslipidemia were investigated as keys to carbohydrate and lipid metabolism disorders.

All patients were clinically free of symptoms except for obesity and they were not taking any medication. Height was measured in all subjects using a standard wall-mounted stadiometer. Weight was measured with a calibrated electronic scale. Anthropometric data also included body mass index (BMI) estimation and waist and hip circumference measurements. BMI was calculated using the weight/height$^2$ (kg/m$^2$) formula. As defined by the National Center for Health Statistics (www.cdc.gov), children with a BMI value above the 95th percentile for age and sex were considered as obese. Relative BMI (relBMI) was calculated using the following formula: subject’s BMI x 100/50$^{th}$ percentile BMI for the subject’s age and sex. Children with a relBMI ≥120 were also accepted as obese (24).

Glucose, lipid and insulin levels were assessed in venous blood following an overnight fast (10-12 h). Serum glucose levels were measured using the glucose hexokinase method. Serum low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride levels were studied using Roche diagnostics methods (GbmH, Germany). Serum insulin levels were measured using the chemiluminescence method (DPC, Los Angeles, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) score was calculated with the following formula: HOMA-IR=fasting serum insulin (µU/mL) x fasting plasma glucose (mmol/L)/22.5 (25). A standard OGTT (1.75 g/kg or a maximum of 75 g of glucose) following a 3-day, high-carbohydrate diet (300 g/day) and a 12-hour overnight fast was performed in 75 obese children. For glucose and insulin assessments, blood samples were obtained at 0, 30, 60, 90 and 120 minutes after glucose administration. Plasma glucose levels were measured with the glucose oxidase method and a modified Trinder color reaction catalyzed by the peroxidase enzyme and insulin levels were measured with an immunoradiometric assay kit.

Glucose tolerance was classified as normal (fasting plasma glucose <100 mg/dL), prediabetes (fasting plasma glucose 100-126 mg/dL and/or impaired glucose tolerance 2-hour postload 140-200 mg/dL) and type 2 diabetes (fasting plasma glucose ≥126 mg/dL and/or 2-hour postload ≥200 mg/dL) (26).

The subjects were defined as IR based on insulin peak of ≥150 µU/mL and/or ≥75 µU/mL 2 hours after a glucose load and when the sum of insulin levels during the OGTT was higher than 300 µU/mL (27,28,29).

The study protocol was approved by the ethics committee of Başkent University and an informed consent from all participants was obtained.

Genotyping: Genomic DNA was prepared from leukocyte pellets by sodium dodecyl sulfate lysis, ammonium acetate extraction and ethanol precipitation. The primers used and the conditions for polymerase chain reaction (PCR) analysis were as described previously (30). The basepair (bp) PCR products were digested with Mval. The uncut product (161 bp) showed the presence of the W allele. When the PCR product was cut into two fragments of 99 and 62 bp, the R allele was revealed.

Data Analysis

Normality of distribution of the continuous variables was analyzed using the Shapiro-Wilk normality test. The Levene’s test was used to assess the homogeneity of variances in the different groups. If parametric test assumptions were available, two independent group means were compared by Student’s t-test. If these assumptions were not available, the Mann-Whitney U test was used for comparison of two group medians. The results were expressed as the number of observations (n) and the mean ± the standard deviation (X±Sx), median (M) and minimum-maximum values. Categorical variables were analyzed by Pearson $\chi^2$ test and Fisher’s exact test when determining the relationships between the variables. Data analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 17.0, SSPS Inc, Chicago IL, USA). A p-value of <0.05 was considered statistically significant.

Results

The clinical characteristics of the study groups are given in Table 1. The screened β-3AR genotypes were not different in the obese and control groups and the frequencies of WW, W/R, R/R genotypes were 88%, 9%, 3% in the obese group and 83%, 16%, 1% in the controls, respectively (p=0.142). The allele frequencies were also similar.

Genotypic distribution satisfied the Hardy-Weinberg equilibrium ($\chi^2$, p=0.9998) in the obese group. One hundred and eight (83.0%) subjects were classified as homozygous
There were no relationships between the polymorphism genotypes and serum fasting glucose, insulin, lipid levels or HOMA scores in obese children (Table 2).

OGTT was performed in 75 obese children. In 4 patients, fasting glucose levels were between 101 and 106 mg/dL. In one patient, the 120-min glucose level was 206 mg/dL and in 11 patients, the 120 min glucose levels were between 140 and 200 mg/dL. 56 of 75 cases had IR. Polymorphism frequency was not different between children who had or did not have IR (p=0.5). Plasma glucose and insulin levels during OGTT were not different between R allele carriers and noncarriers except for 30-minute glucose levels. The mean of the serum glucose level at 30 minutes was significantly higher in the R allele carrier group (Table 3, p=0.027).

### Discussion

Diverse results have been reported in studies on the relation between W64R polymorphism of the β-3AR gene and obesity performed in adults and children. In this study, we investigated the association between W64R polymorphism in the β-3AR gene and both obesity and obesity-related carbohydrate and lipid metabolism disorders in Turkish children. As a further step, we also investigated the relationships between W64R polymorphism of the β-3AR gene and glucose and insulin levels during OGTT in obese children.

The β-3AR gene is involved either directly or indirectly in lipid and glucose metabolism processes and may have an influence on endogenous energy balance and body mass regulation. β-3AR promotes lipolysis and thermogenesis by catecholamine release. Base variation in the β-3AR causes the substitution of the coding sequences from tryptophan into arginine in 64th position and thus influence the affinity of the receptor to norepinephrine. W64R variation of β-3AR is associated with lower metabolic resting rate, with abdominal obesity, weight gain and difficulty losing weight (4,5,8,31,32). However, these findings have not been confirmed in other studies (14,15,33). Interestingly, Genelhu et al (14) found that obese and hypertensive Brazilian adults with W64/W64 genotypes had elevated fasting plasma insulin levels and higher HOMA-IR scores. Such discordant results may be partially explained by ethnicity, age, or population differences in the studied samples. Højlund et al (16) studied W64R genotype of the β-3AR gene in male twins with a high similarity in genetic and environmental background. They found that the heterozygosity for the W64R variant is unlikely to increase the risk of obesity, insulin resistance or type 2 diabetes.

Studies in obese children are also discordant. In some studies with children, similar to our result, R64 allele was not found to be related with obesity (17,18,19). In contrast, some studies showed that the W64R variant was associated with obesity in children (20,21,22). In a recent study, W64R polymorphism was found to be significantly associated with increased visceral fat, dyslipidemia and higher blood pressure in obese children (34).

In the present study, we failed to show any relationship between β-3AR genotype and obesity in children. Also W64R polymorphism of the β-3AR gene was not found to be associated with obesity-related parameters such as insulin resistance, dyslipidemia and hepatosteatosis. This result needs
to be interpreted with caution since the study groups are relatively small in number.

In our study, we have also investigated the association between W64R polymorphism and post-challenge glucose-insulin levels during OGTT. Our results showed that the 30-minute post-challenge glucose levels were significantly higher in obese children who were R allele carriers. Interestingly, fasting glucose, insulin and HOMA scores and other post-challenge glucose or insulin levels in obese children were not different from the controls, except for 30-minute glucose level.

Erhardt et al (34) performed OGTT in obese children but reported no differences in 30-, 60-, 90-, and 120-minute glucose or insulin levels with respect to β-3AR genotypes. Very similar results were also reported in a study in Polish children (35). OGTT has also been often used to evaluate β-cell function and IR (36).

Acute hyperglycemia in response to an oral glucose load has a suppressive effect on endothelium-dependent vasodilatation, leads to an increase in oxidative stress and in the magnitude of the inflammatory response in the vasculature, all of which are processes involved in atherogenesis. Choi et al (37) showed an association between post-challenge 30-minute glucose levels during OGTT and arterial stiffness in Korean adults in whom OGTT was performed to investigate hyperglycemia.

| Table 2. The clinical and laboratory characteristics of obese children with and without R allele |
|-----------------------------------------------|-----------------------------------------------|
| Genotypes (mean ± SD and median; min-max)     | p                                            |
| W64/W64 (n=108)                               | W64/R64 + R64/R64 (n=22)                      |
| Age (years)                                    | 12.2±2.9 (12.0; 35.5-17.8)                    | 13.5±3.2 (13.9; 6.3-17.8) | 0.125 |
| Sex (female/male)                              | 56/52                                        | 12/12                       | 0.818 |
| relBMI (%)                                      | 152.5±24.6 (151; 135.0-240.0)                 | 151.0±20.90 (148.5; 126.0-201.0) | 0.559 |
| Waist/hip ratio                                | 1.0±0.9 (0.9; 0.8-0.9)                        | 0.9±0.1 (0.9; 0.8-1.0)      | 0.276 |
| HDL (mg/dL)                                    | 43.8±1.0 (43.0; 21.0-78.0)                    | 41.0±8.2 (42.0; 25.0-55.0)  | 0.248 |
| LDL (mg/dL)                                    | 96.0±24.8 (99.0; 45.0-163.0)                  | 96.9±18.4 (92.5; 74.0-133.0) | 0.251 |
| Triglyceride (mg/dL)                           | 112.8±60.9 (104.3; 42-460.0)                  | 115.4±47.2 (107.5; 48.0-224.0) | 0.504 |
| Fasting glucose (mg/dL)                       | 87.8±10.0 (88.0; 77.0-116.0)                  | 87.3±7.5 (89.5; 74.0-99.0)  | 0.839 |
| Fasting insulin (µU/mL)                       | 18.7±13.7 (15.5; 2.0-87.0)                    | 14.5±7.53 (13.8; 2.7-33.0)  | 0.210 |
| HOMA score                                     | 3.8±2.8 (3.2; 0-19.98)                        | 3.16±1.73 (3.15; 0.49-7.58) | 0.390 |

SD: standard deviation, BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA: homeostatic model assessment

| Table 3. Glucose and lipid levels during oral glucose tolerance test (OGTT) in R allele carriers and non-carrier patients |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Variables                                             | W64/ W64 (n=60)       | W64/R64 + R64/R64 (n=15) |
| Glucose 0                                             | 89.8±7.9 (90.6; 7.0-116.0)       | 88.1±7.3 (90.0; 74.0-99.0)  | 0.526 |
| Insulin 0                                              | 22.1±13.1 (18.9; 3.0-87.0)       | 16.9±7.5 (17.0; 6.6-35.0)   | 0.113 |
| Glucose 30                                             | 137.5±23.6 (135.5; 91.0-196.0)    | 153.2±26.6 (145; 114.0-202.0) | 0.027 |
| Insulin 30                                             | 133.68±84.23 (106.50; 6.15-406.0) | 133.22±54.22 (136.1; 52.0-226.0) | 0.686 |
| Glucose 60                                             | 140.0±28.9 (131.0; 80.0-214.0)    | 135.6±15.9 (131.0; 120.0-174.0) | 0.942 |
| Insulin 60                                             | 142.7±100.9 (119.4; 16.1-472.0)   | 139.5±83.3 (124.0; 39.0-330.0) | 0.848 |
| Glucose 90                                             | 130.0±26.4 (124.5; 88.0-222.0)    | 121.1±17.9 (116.0; 100.0-152.0) | 0.251 |
| Insulin 90                                             | 139.4±113.8 (99.0; 19.6-548.0)    | 108.3±64.2 (90.0; 40.4-300.0) | 0.591 |
| Glucose 120                                            | 121.0±24.9 (117.5; 75.0-206.0)    | 116.9±17.1 (113.0; 84.0-146.0) | 0.730 |
| Insulin 120                                            | 141.4±121.0 (100.0; 10.2-609.0)   | 125.2±99.8 (102.0; 26.1-350.0) | 0.662 |
| Mean insulin                                           | 112.5±33.2 (104.4; 22.0-383.0)    | 104.8±52.6 (95; 38.0-245.0)  | 0.633 |
| Peak insulin                                           | 197.0±119.0 (168.0; 26.0-609.0)   | 177.5±85.7 (148.0; 52.0-350.0) | 0.702 |
| Sum of insulin                                         | 565.0±318.5 (522.0; 110.0-1917.0) | 991.1±1897.9 (475.0; 182.0-7790.0) | 0.708 |

All glucose levels were expressed as mg/dL and insulin levels as µU/mL.
Urine albumin excretion is a marker for vascular damage. It has been shown that the 30-minute post-challenge plasma glucose level is associated with urine albumin excretion in males and in postmenopausal women with normal glucose regulation. Besides its effect on lipolysis and biological energy production, \( \beta \)-3AR may modulate peripheral vascular tone and increase the blood pressure (38). Some clinical studies pointed to a possible relationship between arterial hypertension and \( \beta \)64R polymorphism of the \( \alpha \)DRB3 gene as well as to a relationship between this genotype and higher mortality among hypertensive patients (9,13). In another study, obesity and hypertension have been considered to be related to polymorphisms of the \( \beta \)-3AR gene (4). An important limitation of our study is that we have no data about the blood pressure levels of our patients.

In conclusion, our results showed that among obese children, R allele carriers have higher post-challenge 30-minute glucose levels. Our results warrant further support from studies on relationships between \( \beta \)-3AR polymorphism and acute glucose excursions and vascular tone impairment in obesity. Our findings may be a step in the clarification of the big puzzle of glucose metabolism and also of hypertension. Further research is needed to identify \( \beta \)64R polymorphism as a new view of glucose metabolism and also of hypertension. Further these children might help us to understand the interactions of the molecular basis of obesity. Long-term follow-up of our findings may be a step in the clarification of the big puzzle of glucose metabolism and vascular tone impairment in obesity.

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References

1. Maffeis C. Aetiology of overweight and obesity in children and adolescents. Eur J Pediatr 2000;159(Suppl 1):35-44.
2. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, R, Schull WJ, Schulsinger F. An adoption study of human obesity. N Engl J Med 1986;314:193-198.
3. Stunkard AJ, Sørensen TI, Hanis C, Teasdale TW, Chakraborty R, Castro MJ. Interaction of -55CT polymorphism of UCP3 gene with Trp64Arg polymorphism of beta3adrenoceptor gene on insulin resistance in obese patients. Eur Rev Med Pharmacol Sci 2012;16:610-616.
4. Mirrakhimov AE, Keremikulova AS, Lunegova OS, Moldokeeva CB, Zalesskaya YY, Ablitova SS, Sohovzova NA, Aldasheva AA, Mirrakhimov EM. An association between TRP64ARG polymorphism of the B3 adrenoceptor gene and some metabolic disturbances. Cardiovasc Diabetol 2011:10.89.
5. Masuo K, Lambert GW. Relationships of adrenoceptor polymorphisms with obesity. J Obes 2011;2011:609485. Epub 2011 Apr 4
6. Masuo K. Roles of beta2- and beta3-adrenoceptor polymorphisms in hypertension and metabolic syndrome. Int J Hypertens 2010;2010:832681.
7. Wang Y, Luk AO, Ma RC, So WY, Tam CH, Ng MC, Yang X, Baum L, Lam V, Tong PC, Chan JC. Independent predictive roles of eotaxin, Ala23Thr, paraoxonase 2 Ser311Cys and beta-adrenergic receptor Trp64Arg polymorphisms on cardiac disease in Type 2 Diabetes—an 8-year prospective cohort analysis of 1297 patients. Diabet Med 2010;27:376-383.
8. Tsunekawa K, Yanagawa Y, Akoh T, Morimura T, Araki O, Ogawa T, Kawai Y, Mitani Y, Lezhava A, Yanagawa M, Hayashizaki Y, Murakami M. Association between accumulation of visceral fat and the combination of \( \beta \)-adrenergic receptor Trp64Arg, \( \beta \)2 adrenergic receptor Arg16Gly and uncoupling protein 1-3826A>G polymorphisms detected by Smart Amplification Process 2. Endocr J 2011;58:1079-1086.
9. Iwamoto Y, Ohishi M, Yuan M, Tatar A, Kato N, Takeya Y, Onishi M, Maekawa Y, Kamide K, Rakugi H. \( \beta \)-Adrenergic receptor gene polymorphism is a genetic risk factor for cardiovascular disease: a cohort study with hypertensive patients. Hypertens Res 2011;34:573-577. Epub 2011 Feb 3
10. de Luis DA, Alber R, Izola O, Gonzalez Sagrado M, Conde R, Castro MJ. Polymorphism of Trp64Arg of beta3adrenoceptor gene in obese patients. Eur J Pediatr 2000;159(Suppl 1):35-44.
11. Lin SY, Shue WH, Lee WJ, Song YM, Chen YT. Trp64Arg polymorphism of the beta 3-adrenergic receptor gene is not associated with obesity in Chinese women. Zhonghua Yi Xue Za Zhi (Taipei) 1999;62:569-576.
12. Tafel J, Branscheid I, Skwarna B, Schönme M, Morcos M, Algenstaedt P, Hinney A, Hefenbrock J, Newroth P, Hamann A, Variants in the human \( \beta \)3-adrenergic receptor and melanocortin-4 receptor genes are not associated with morbid obesity in children and adolescents. Diabetes Obes Metab 2004;6:452-455.
13. Kinoshita T, Hanaki K, Nagaiishi J, Kawashima Y, Adachi K, Nanba E, Kanzaki S. Variation analysis of \( \beta \)3-adrenergic receptor gene on insulin resistance in obese patients. Eur Review Med Pharmacol Sci 2011:16:610-616.
21. Arashiro R, Katsuren K, Fukuyama S, Ohta T. Effect of Trp64Arg mutation of the beta3-adrenergic receptor gene and C161T substitution of the peroxisome proliferator activated receptor gamma gene on obesity in Japanese children. Pediatr Int 2003;45:135-141.

22. Porto PI, Garcia SI, Dieuzeide G, González C, Landa MS, Pirola CJ. Clinical features of the metabolic syndrome in adolescents: minor role of the Trp64Arg beta3-adrenergic receptor gene variant. Pediatr Res 2004;55:836-841.

23. Oguri K, Tachi T, Matsuoka T. Visceral fat accumulation and metabolic syndrome in children: the impact of Trp64Arg polymorphism of the beta3-adrenergic receptor gene. Acta Paediatr 2013;102:613-619. Epub 2013 Jan 22

24. Bundak R, Furman A, Guncz H, Darendeliler F, Bas F, Neyzi O. Body mass index references for Turkish children. Acta Paediatr 2006;95:194-198.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and -cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-419.

26. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;31(Suppl 1):55-60.

27. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment: insulin resistance and -cell function from fasting plasma glucose and insulin concentrations in man. Diabetes Care 1985;28:412-419.

28. Reaven GM, Chen YD, Hollenbeck CB, Sheu WH, Ostrega D, Polonsky KS. Plasma insulin, C-peptide, and proinsulin concentrations in obese and nonobese individuals with varying degrees of glucose tolerance. J Clin Endocrinol Metab 1993;76:44-48.

29. Zawodniak-Szaβapska M, Stawerska R, Brzeziacska E, Pastuszak-Lewandoska D, Lukomowicz J, Cypryk K, Lewi ski A. Association of Trp64Arg polymorphism of beta3-adrenergic receptor with insulin resistance in Polish children with obesity. J Pediatr Endocrinol Metab 2008;21:147-154.

30. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing. Comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-1470.

31. Choi ES, Rhee EJ, Choi JH, Bae JC, Yoo SH, Kim WJ, Park SE, Park CY, Lee WY, Cho YK, Oh KW, Park SW, Kim SW. The association of brachial-ankle pulse wave velocity with 30-minute post-challenge plasma glucose levels in Korean adults with no history of type 2 diabetes. Korean Diabetes J 2010;34:287-293. Epub 2010 Oct 31

32. Li D, Hou X, Ma X, Zong W, Lu H, Xiang K, Jia W. Association between an increment of 30-minute postchallenge plasma glucose and urine albumin excretion exists in postmenopausal women but not in premenopausal women. Menopause 2011;18:1303-1308.