**Original Article**
EPIDEMIOLOGY/GENETICS

Obesity

# Genetic Association of Adrenergic Receptor Alpha 2a with Obesity and Type 2 Diabetes

*Ewa-Carin Långberg, Mohammed Seed Ahmed, Suad Efendic, Harvest F. Gu and Claes-Göran Östenson*

**Objective:** The sympathetic nervous system (SNS) is linked to glucose, lipid, and protein metabolism. The $\alpha_{2A}$-adrenergic receptor (ADRA2A) is involved in the SNS and mediates inhibition of insulin secretion and lipolysis. The association of *ADRA2A* single-nucleotide polymorphisms (SNPs) with obesity and/or type 2 diabetes (T2D) was investigated.

**Design and Methods:** Genotyping was performed in a case–control study of 1,177 Swedish individuals, including lean and obese subjects with normal glucose tolerance (NGT) and T2D patients. *ADRA2A* mRNA expression was measured in pancreatic islets isolated from T2D patients and nondiabetic subjects.

**Results:** SNP rs553668 was associated with T2D in men (odds ratio [OR] = 1.47; 95% confidence interval [CI] = 1.08–2.01; $P = 0.015$) but this association was lost after adjusting for age and for body mass index (BMI). Associations were also detected when comparing obese NGT and lean NGT subjects (OR = 1.49; 95% CI = 1.07–2.07; $P = 0.017$), and in obese (OR = 1.62; 95% CI = 1.06–2.49; $P = 0.026$), but not in lean T2D. In women, multiple logistic regression regarding SNP rs521674 demonstrated an increased OR of 7.61 (95% CI = 1.70–34.17; $P = 0.008$) for T2D when including age as a covariant. Correcting for BMI removed the significant association. When age was included in the model, association also found when obese T2D patients were compared with lean NGT subjects ($P = 0.041$). *ADRA2A* mRNA expression in human pancreatic islets was detectable, but with no statistically significant difference between the diabetic and the control groups.

**Conclusions:** *ADRA2A* genetic polymorphisms are mainly associated with obesity and possibly with T2D in a Swedish population.

*Obesity* (2013) **21**, 1720-1725. doi:10.1002/oby.20162

## Introduction

Type 2 diabetes (T2D) is often linked to obesity, and both T2D and obesity are thought to be caused by an interaction between genetic and environmental factors. As to heredity, the identification of susceptibility genes is of great importance for prevention and future therapeutic applications.

The sympathetic nervous system (SNS) plays an important role in regulating metabolism of glucose and lipids (1). In addition to direct effects on metabolic substrate fluxes, SNS modulates release of insulin and glucagon which in turn regulates metabolism of glucose, lipids, and protein. Adrenaline increases glucose levels by stimulating glycogenolysis and by decreasing glucose clearance by β-adrenergic mechanisms as well as by inhibiting insulin release and stimulating glucagon secretion via α-adrenergic action (1). Catecholamines are the most important regulators of lipolysis in human adipose tissue (2). Thus, stimulation of $\beta_2$-adrenergic receptors enhances lipolysis, whereas stimulation of $\alpha_2$-adrenergic receptors inhibits lipolysis (2). Insulin inhibits catecholamine-stimulated lipolysis by reducing the effects of adrenaline on $\beta_2$- and stimulating $\alpha_2$-adrenergic receptors in adipocytes (3).

Recent data indicate that obesity is characterized by activation of the SNS (4). The increased sympathetic tone in obese individuals may have adverse effects on pancreatic function and contribute to the abnormal glucose-induced insulin secretion in obese subjects (5). Increased activity of the SNS also contributes to development of hypertension and elevated cardiovascular risk in obese subjects (6).

Department of Molecular Medicine and Surgery, Rolf Luft Center for Diabetes Research, Karolinska Institutet, Karolinska University Hospital (Solna), Stockholm, Sweden. Correspondence: Claes-Göran Östenson (claes.ostensson@karolinska.se)

**E.-C. Långberg and M. Seed Ahmed have contributed equally to this work.**
**Disclosure:** The authors declared no conflicts of interest.
**Funding agencies:** This research was supported by The Swedish Research Council, The Swedish Diabetes Association, Novo Nordisk Scandinavia, Novo Nordisk Consortium and The Loo and Hans Osterman Foundation.
**Received:** 12 July 2011 **Accepted:** 1 November 2012 **Published online 19 November 2012. doi:10.1002/oby.20162**

As described above, catecholamines exert an important physiological role by $\alpha_2$-adrenergic receptor-mediated inhibition of insulin secretion in animals and in man. In contrast, stimulation of $\beta_2$-adrenergic receptors enhances insulin release (7). Increased expression of $\alpha_2$-adrenergic receptors in β-cells can cause alterations in insulin secretion regulation and contribute to etiology of T2D (8). $\beta_{2A}$-Adrenergic receptor-deficient mice exhibit increased plasma insulin levels, reduced blood glucose levels, and improved glucose tolerance (9).

Polymorphisms in the human $\alpha_{2A}$-adrenergic receptor (*ADRA2A*) gene have been identified and associated with obesity (10,11), elevated glucose levels (12), hypertension (13), and cardiovascular diseases (14). Recently, it was reported that a polymorphism in the *ADRA2A* gene is associated with reduced insulin secretion and increased risk of T2D (15). The intention of our study was to investigate the role of *ADRA2A* genetic variants in human T2D and/or obesity. We have genotyped single-nucleotide polymorphisms (SNPs) in the *ADRA2A* gene in a Swedish cohort, including lean healthy controls, obese subjects, and T2D patients. We have also studied *ADRA2A* mRNA expression in pancreatic islets isolated from T2D patients and nondiabetic subjects.

# Subjects and Methods

## Subjects

A total of 1,177 individuals were included in this study and all of them were selected from the Stockholm Diabetes Prevention Program (SDPP) cohort investigated in both a baseline study and a follow-up study 8–10 years later, as described previously (16). The individuals are unrelated and of Swedish origin. All subjects classified as T2D within the SDPP cohort were selected: newly diagnosed in the baseline examination, newly diagnosed in the follow-up examination, or diagnosed between baseline and follow-up, in total of 399 subjects (235 men and 164 women). Their body mass index (BMI) ranged from 18.4 to 58.6 kg/m$^2$. In addition, all men who had normal glucose tolerance (NGT) and BMI of $\geq$30.0 kg/m$^2$ at baseline and did not develop neither prediabetes (impaired fasting glucose/impaired glucose tolerance) nor T2D at follow-up (=obese NGT), were selected, $n$ = 198. We did not have access to samples from obese women with NGT. Control subjects (= lean NGT) were randomly selected from individuals with NGT at both baseline and follow-up, with a BMI of $\leq$26 kg/m$^2$ and without family history of diabetes. Thus, 580 subjects were selected (394 men and 186 women) corresponding to 73% of eligible men ($n$ = 542) and to 24% of eligible women ($n$ = 787). T2D patients were diagnosed according to criteria from WHO 1998 and the standard definition of obesity was used according to the Center for Disease Control (CDC, 1998) (17,18). For T2D patients diagnosed between baseline and follow-up studies, baseline data were used to avoid the influence of lifestyle changes and/or antidiabetic treatment on phenotypes. Clinical data on included patients and subjects are summarized in Table 1. All participants gave their informed consent to take part in the study. Procedures followed were in concordance with the declaration of Helsinki II and approved by the local ethics committee.

## Human islets

With support from the Nordic Network for Clinical Islet Transplantation (head coordinator Prof. Olle Korsgren, Department of Clinical Immunology, Uppsala University, Sweden), human pancreatic islets were isolated from brain-dead, heart-beating, multiorgan donors. In all, islets were isolated from 24 donors (16 patients with T2D and 8 nondiabetic controls). The procedure of islet isolation is a refinement of the automated method for isolation of human pancreatic islets (19). The study was approved by the Human Research Ethics Committee of Karolinska Institutet.

## Genotyping

DNA was extracted from peripheral blood of SDPP individuals using Puregene DNA purification kit (Gentra Systems, Minneapolis, MN). Three SNPs were chosen based on the location, function, and previous validation. The selected SNPs were picked to cover the whole gene. Tagger® program from the International HapMap

**TABLE 1** Clinical characteristics of subjects

| Clinical parameters | NGT lean subjects | NGT obese subjects | T2D subjects |
|---|---|---|---|
| *n* (Men/women) | 580 (394/186) | 198 (198/–) | 399 (235/164) |
| Age (years) | 47 (46–47) | 46 (46–47) | 52 (52–53) |
| BMI (kg/m$^2$) | 22.9 (22.8–23.1) | 32.2 (31.9–32.5) | 30.1 (29.6–30.7) |
| Waist (cm) | 82.2 (81.6–82.8) | 104.9 (103.9–105.9) | 98.4 (97.2–99.7) |
| Waist-hip ratio | 0.84 (0.84–0.85) | 0.95 (0.94–0.95) | 0.92 (0.92–0.93) |
| Fasting plasma glucose (mmol/l)[a] | 4.5 (4.4–4.5) | 4.8 (4.7–4.8) | 6.5 (6.4–6.7) |
| 2-h Plasma glucose (mmol/l)[a] | 4.0 (3.9–4.1) | 4.8 (4.6–5.0) | 10.5 (10.1–11.0) |
| Fasting plasma insulin (mU/l)[a] | 13.0 (12.5–13.5) | 24.1 (22.8–25.5) | 23.1 (21.9–24.3) |
| 2h Plasma insulin (mU/l)[a] | 33.6 (32.2–35.1) | 62.9 (57.3–69.1) | 81.8 (76.7–87.3) |
| HOMA-IR index[a] | 2.6 (2.5–2.7) | 5.1 (4.8–5.4) | 6.7 (6.3–7.1) |
| Systolic BP (mmHg) | 119 (118–120) | 131 (129–133) | 139 (137–141) |
| Diastolic BP (mmHg) | 75 (74–76) | 84 (82–85) | 85 (84–86) |

Data are means (95% CI) for normally distributed variables.
Insulin resistance index was calculated by HOMA.
NGT, normal glucose tolerance; T2D, type 2 diabetes; BMI, body mass index; BP, blood pressure; IR, insulin resistance.
[a]Geometric means (95% CI) for logarithmic transformed variables.

project (release no. 27) was also used to select and evaluate tagSNPs from genotype data in HapMap (20). Here, pair-wise tagging was used together with an $r^2$ cutoff of 0.8 and a minor allele frequency (MAF) of 5%. Two SNPs in the *ADRA2A* gene (rs11195419 and rs553668) were captured by Tagger based on HapMap data. The genotyped SNPs captured the HapMap tagging SNPs.

To confirm the selected SNP genotyping assays in our population, we used 32 Swedish DNA samples including 16 cases and 16 NGT controls. All studied SNPs had at least 5% allele frequency and were used for further genotyping in the larger sample set. High-throughput genotyping was performed using TaqMan allelic discrimination (ABI 7300, Applied Biosystems, USA). Information on primer and probe sequences used are available, upon request. All PCR reactions were run in 20 µl volumes using 10–20 ng genomic DNA. For genotyping quality control, samples were checked by rerunning a random set of 100 samples and comparing them against the original genotypes. The genotyping success rate was 99%. All genotypes were read by two independent investigators who did not have access to the phenotypic status of the study objects.

### mRNA expression

Total RNA was extracted from human islets using RNeasy mini kit, following the manufacturer's protocol (Qiagen, Hilden, Germany). Islets were disrupted using a Mini Beadbeater (Biospec products, Bartlesville, OK) with 500 µl (2 mm) Zirconia beads. cDNA transcription was performed using QuantiTect Reverse Transcription kit (Qiagen, Valencia, Germany). Real-time RT-PCR was performed with TaqMan gene expression assay in a ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA).

### Statistics

Hardy–Weinberg equilibrium was tested by $\chi^2$-test (21). The powers to detect a clinically relevant increased risk of 40% per risk allele for T2D were 61, 76, and 89%, assuming minor allele frequencies of 0.10, 0.15, and 0.24, respectively, and the prevalence of T2D to be 6% and sample sizes as in this study (22). Data are presented as means (95% CI) or geometric means (95% CI) in Table 1 and as means ± SE for *ADRA2A* mRNA expression data from isolated human pancreatic islets. Normal probability plots were created and parameter distributions were transformed to natural logarithm when needed to improve skewness and to obtain a normal distribution before performing statistical analysis. Homogeneity of variances was tested by Levene's test. Allele distributions were compared between cases (T2D patients and NGT obese subjects) and NGT control subjects and odds ratios (ORs), and 95% confidence intervals (CIs) were calculated to test for associations. Multiple logistic regression analysis, with results expressed as OR with 95% CI, was used to assess whether the risk alleles were associated with T2D and/or obesity after adjustments for age and also for body measurements when appropriate. Comparisons were made in men and women separately. The homeostasis model assessment was used to assess insulin resistance (HOMA-IR). Based on the fasting glucose and insulin levels, it was calculated according to the following equation: fasting plasma glucose (mmol/l) × fasting plasma insulin (mU/ml)/22.5 (23). Independent *t*-test was used to analyze data obtained from the human islets. *P*-values of $\leq 0.05$ were considered significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC) and PASW version 18 (SPSS, Chicago, IL) programs.

## Results

We genotyped three SNPs in the *ADRA2A* gene in our SDPP individuals and genotype distributions of all studied SNPs followed by Hardy–Weinberg equilibrium. Table 2 provides a list of studied SNPs and their minor allele frequencies. Test for associations in all SNPs was performed. Comparison analyses were carried out in men and women separately. We found associations linked to two of the studied SNPs, rs553668 and rs521674. The results will be focused on these two SNPs as rs11195419 did not show significant allelic association. We also performed gene expression analysis of *ADRA2A* in pancreatic islets of 24 human donors.

### SNP rs553668

SNP rs553668 in the *ADRA2A* gene showed significant association between T2D patients and lean NGT subjects in men (OR = 1.47; 95% CI = 1.08–2.01; $P = 0.015$; Table 3). Carriers with the A/A genotype had an increased risk of disease. However, male T2D patients had BMI values ranging from 18.4 to 45.6 kg/m$^2$. To determine whether this polymorphism is associated with T2D or with obesity, we analyzed obese NGT and lean NGT subjects and found an association of the A allele with obesity (OR = 1.49; 95% CI = 1.07–2.07; $P = 0.017$). We also compared lean T2D patients with lean NGT subjects and no association was found (OR = 1.09; 95% CI = 0.61–1.95; $P = 0.780$). However, when comparing obese T2D male patients with lean NGT male subjects, there was a significant difference (OR = 1.62; 95% CI = 1.06–2.49; $P = 0.026$). No associations were seen when comparing obese T2D and obese NGT (OR = 1.09; 95% CI = 0.69–1.72; $P = 0.709$) or lean and obese T2D versus lean and obese NGT (OR = 1.22; 95% CI = 0.86–1.73; $P = 0.265$). Comparisons of rs553668 in men are summarized in Table 3.

Multiple logistic regression analysis for SNP rs553668, including age in the model and comparing T2D and lean NGT men, showed that A/A genotype carriers had an increased odds ratio compared to G/G genotype carriers (OR = 3.09; 95% CI = 0.99–9.59; $P = 0.051$, Table 3). When we included BMI in the model, the association disappeared (OR = 2.29; 95% CI = 0.34–15.49; $P = 0.394$). We then compared obese NGT and lean NGT groups and detected a significant association (OR = 2.82; 95% CI = 1.03–7.74; $P = 0.044$). There was no significant association when comparing lean T2D and lean NGT (OR = 1.76; 95% CI = 0.25–12.58; $P = 0.572$) but when looking at obese T2D and lean NGT subjects we found a significant association (OR = 4.17; 95% CI = 1.02–17.00; $P = 0.046$). No associations were found with obese T2D versus obese NGT groups (OR = 1.10; 95% CI = 0.30–4.07; $P = 0.889$) or between lean and obese T2D compared with lean and obese NGT (OR = 1.69; 95% CI = 0.58–4.95; $P = 0.339$). Multiple logistic

**TABLE 2** Studied SNPs in the *ADRA2A* gene and their MAFs in Swedish population

| Gene | SNP ID | SNP location | SNP type | MAF |
|---|---|---|---|---|
| **ADRA2A** | rs521674 | 5′-Upstream | W = A/T | 0.24 |
| **ADRA2A** | rs11195419 | 3′-UTR | M = A/C | 0.10 |
| **ADRA2A** | rs553668 | 3′-UTR | R = A/G | 0.15 |

MAF, minor allele frequency; UTR, untranslated region.

**TABLE 3** SNP rs553668: genotype distribution and association in men

| Comparisons | Genotype distribution | | | MAF | Allele frequency difference | | | Multiple logistic regression | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | G/G[a] | G/A | | | A/A | | |
| | G/G *n* (%) | G/A *n* (%) | A/A *n* (%) | | OR | 95% CI | *P*-value | OR | OR | 95% CI | *P* | OR | 95% CI | *P*-value |
| **T2D vs. lean NGT** | 158/295 (35/65) | 65/89 (42/58) | 10/7 (59/41) | 0.18/0.13 | 1.47 | 1.08–2.01 | 0.015 | 1.00 | 1.26 | 0.82–1.92 | 0.295[b] | 3.09 | 0.99–9.59 | 0.051[b] |
| **T2D vs. lean NGT** | | | | | | | | 1.00 | 0.96 | 0.47–1.96 | 0.913[c] | 2.29 | 0.34–15.49 | 0.394[c] |
| **Obese NGT vs. lean NGT** | 134/295 (31/69) | 55/89 (38/62) | 9/7 (56/44) | 0.18/0.13 | 1.49 | 1.07–2.07 | 0.017 | 1.00 | 1.35 | 0.91–2.01 | 0.132[b] | 2.82 | 1.03–7.74 | 0.044[b] |
| **Lean T2D vs. lean NGT** | 40/295 (12/88) | 11/89 (11/89) | 2/7 (22/78) | 0.14/0.13 | 1.09 | 0.61–1.95 | 0.780 | 1.00 | 0.97 | 0.46–2.06 | 0.934[b] | 1.76 | 0.25–12.58 | 0.572[b] |
| **Obese T2D vs. lean NGT** | 56/295 (16/84) | 26/89 (23/77) | 4/7 (36/64) | 0.20/0.13 | 1.62 | 1.06–2.49 | 0.026 | 1.00 | 1.35 | 0.75–2.43 | 0.319[b] | 4.17 | 1.02–17.00 | 0.046[b] |
| **Obese T2D vs. obese NGT** | 56/134 (29/71) | 26/55 (32/68) | 4/9 (31/69) | 0.20/0.18 | 1.09 | 0.69–1.72 | 0.709 | 1.00 | 0.83 | 0.44–1.57 | 0.558[b] | 1.10 | 0.30–4.07 | 0.889[b] |
| **Lean + obese T2D vs. lean + obese NGT** | 96/429 (18/82) | 37/144 (20/80) | 6/16 (27/73) | 0.18/0.15 | 1.22 | 0.86–1.73 | 0.265 | 1.00 | 1.02 | 0.64–1.63 | 0.943[b] | 1.69 | 0.58–4.95 | 0.339[b] |

MAF: minor allele frequency.
Genotype data on SNP rs553668 are missing for two subjects with T2D and three lean subjects with NGT.
[a]Reference group.
[b]Adjusted for age.
[c]Adjusted for age and BMI.

regression analysis for G/A genotype carriers compared with G/G genotype carriers in the SNP rs553668 in men showed no significant results (Table 3).

### SNP rs521674

SNP rs521674 revealed a tendency of association comparing T2D patients and lean NGT subjects in women (OR = 1.40; 95% CI = 0.97–2.03; $P = 0.072$) (Table 4). The T2D group in women includes subjects with BMI ranging from 19.7 to 58.6. To determine if a possible association is linked to T2D alone or if it is reflecting obesity, we analyzed lean T2D patients versus lean NGT subjects. No association was found (OR = 1.13; 95% CI = 0.62–2.07; $P = 0.687$).

Multiple logistic regression analysis was carried out for the above SNP in women. When including age in the model, comparing T2D patients with lean NGT subjects showed that A/A genotype carriers had an increased ORs compared with T/T genotype carriers (OR = 7.61; 95% CI = 1.70–34.17; $P = 0.008$) (Table 4). Adding BMI into the model changed the outcome, and thus rs521674 was no longer associated with T2D (OR = 2.66; 95% CI = 0.43–16.61; $P = 0.296$). Comparing lean T2D and lean NGT groups did not show any association (OR = 2.23; 95% CI = 0.36–13.83; $P = 0.391$) although we detected a risk in obese T2D versus lean NGT (OR = 10.89; 95% CI = 1.11–107.10; $P = 0.041$). We also found an association between lean and obese T2D versus lean NGT (OR = 5.10; 95% CI = 1.13–22.98; $P = 0.034$). With regard to A/T genotype carriers compared with T/T genotype carriers in the SNP rs521674 in women, multiple logistic regression analysis including age in the model revealed a significant increase in the OR when comparing T2D patients with lean NGT subjects (OR = 6.35; 95% CI = 1.39–29.10; $P = 0.017$). Adding BMI into the model removed the association (OR = 3.89; 95% CI = 0.58–20.19; $P = 0.163$). There was no significant association when comparing lean T2D and lean NGT (OR = 2.28; 95% CI = 0.35–14.77; $P = 0.389$) but when looking at obese T2D and lean NGT subjects we found a significant association (OR = 10.50; 95% CI = 1.06–104.21; $P = 0.045$). Moreover, we detected an association between lean and obese T2D versus lean NGT (OR = 4.91; 95% CI = 1.07–22.50; $P = 0.040$).

### *ADRA2A* mRNA

Although *ADRA2A* mRNA expression (mean ± SE) was detectable in isolated human pancreatic islets, no statistically significant difference was found between the diabetic and the control groups (0.35 ± 0.15 vs. 0.99 ± 0.54 arbitrary units; $P = 0.209$).

## Discussion

We have evaluated genetic association for the *ADRA2A* gene with obesity and/or T2D in a Swedish cohort. In addition, we have investigated *ADRA2A* mRNA expression in isolated human pancreatic islets. Data indicate that SNP rs553668 is mainly associated with obesity in men. For women, SNP rs521674 might be associated with obesity and possibly also with T2D. Our data support the notion that *ADRA2A* genetic variants play an important role in the pathogenesis of obesity and they might also modify progression to T2D.

SNP rs553668 has been reported to be associated with risk of obesity in Caucasians and African Americans (10–12), hypertension in

**TABLE 4** SNP rs521674: genotype distribution and association in women

| Comparisons | Genotype distribution | | | MAF | Allele frequency difference | | | Multiple logistic regression | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | T/T[a] | A/T | | | A/A | | |
| | A/A *n* (%) | A/T *n* (%) | T/T *n* (%) | | OR | 95% CI | *P*-value | OR | OR | 95% CI | *P*-value | OR | 95% CI | *P*-value |
| **T2D vs. lean NGT** | 103/106 (49/51) | 54/60 (47/53) | 3/14 (18/82) | 0.19/0.24 | 1.40 | 0.97–2.03 | 0.072 | 1.00 | 6.35 | 1.39–29.10 | 0.017[b] | 7.61 | 1.70–34.17 | 0.008[b] |
| **T2D vs. lean NGT** | | | | | | | | 1.00 | 3.89 | 0.58–20.19 | 0.163[c] | 2.66 | 0.43–16.61 | 0.296[c] |
| **Lean T2D vs. lean NGT** | 22/106 (17/83) | 12/60 (17/83) | 2/14 (13/87) | 0.22/0.24 | 1.13 | 0.62–2.07 | 0.687 | 1.00 | 2.28 | 0.35–14.77 | 0.389[b] | 2.23 | 0.36–13.83 | 0.391[b] |
| **Obese T2D vs. lean NGT** | 46/106 (30/70) | 32/60 (35/65) | 1/14 (7/93) | 0.22/0.24 | 1.18 | 0.75–1.85 | 0.470 | 1.00 | 10.50 | 1.06–104.21 | 0.045[b] | 10.89 | 1.11–107.10 | 0.041[b] |
| **Lean + obese T2D vs. lean NGT** | 68/106 (39/61) | 44/60 (42/58) | 3/14 (18/82) | 0.22/0.24 | 1.17 | 0.79–1.73 | 0.449 | 1.00 | 4.91 | 1.07–22.50 | 0.040[b] | 5.10 | 1.13–22.98 | 0.034[b] |

MAF: minor allele frequency.
Genotype data on SNP rs521674 are missing for four subjects with T2D and six lean subjects with NGT.
[a]Reference group.
[b]Adjusted for age.
[c]Asjusted for age and BMI.

African Americans (14), and with endurance in athletes of Caucasian origin (24). This polymorphism has also been associated with platelet aggregation (25). Our study confirms the association of SNP rs553668 with obesity in Swedish men. This SNP is a tagSNP and located in the 3′-region of the *ADRA2A* gene. Genetic variations in the 3′-region is important in the regulation of message stability. Alteration in this region of the *ADRA2A* gene can affect gene expression by causing an increased stability of the mRNA (26).

After accomplishing our study, it was reported that rs553668 is associated with reduced insulin secretion and increased risk of T2D in a population from Finland and southern Sweden (15). This association was verified on functional level in human pancreatic islets. Risk allele carriers showed overexpression of *ADRA2A* in islets, decreased insulin secretion, and reduced number of docked insulin granules *in vitro*. These effects were corrected by ADRA2A-antagonism (15). Although *ADRA2A* mRNA expression was detectable in our isolated human pancreatic islets, no significant difference was found between the diabetic and the control groups. These divergent results may be explained by the fact that T2D is a complex disease with many different entities and involvement of multiple genes as well as environmental factors. Also, in the study by Rosengren et al., the individuals were not categorized in diabetic and control groups. This was taken into consideration in our study. We propose that *ADRA2A* polymorphisms are important components in obesity and possibly in T2D occurrence, which may allow future specific therapy for individual patients and personalized medicine.

SNP rs521674 is situated in the 5′-region of the *ADRA2A* gene. Owing to its location, this polymorphism has been previously described and used as a genetic marker (27,28). Genetic variants found in the 5′-flanking region could be involved in transcription binding sites in the promoter region and influence gene expression. The responsiveness to $\alpha_2$-adrenergic stimulation differs markedly between individuals. This could be owing to receptor regulation processes (29,30) or to hereditary interindividual variances in $\alpha_2$-adrenoreceptor responsiveness (31). There is little information on *in vivo* effects of studied SNPs in the *ADRA2A* gene. The variants found are mainly noncoding and there is limited knowledge on their functional significance. Our results suggest that SNP rs521674 might be associated with obesity and possibly also with T2D. The association was seen in obese and not lean T2D when age was included in the multiple logistic regression model. We did not have access to samples from obese women with NGT and therefore could not compare this group with lean women with NGT. We found association between the combined group of lean and the obese T2D patients and lean NGT subjects after adjusting for age and were not able to exclude a link to T2D.

Gender seems to be an important factor to take into account when analyzing genetics of obesity and related disorders. We and others have previously demonstrated differences in other genes among men and women in associations of SNPs with obesity and T2D. These genes include receptor protein tyrosine phosphatase sigma (*RPTPσ*) (32), angiotensin-converting enzyme (*ACE*) (33), low-density lipoprotein-related protein-associated protein 1 (*LRPAP1*), thrombospondin I (*THBS1*), acetyl-coenzyme A acetyltransferase 2 (*ACAT2*), integrin β 3 (*ITGB3*), coagulation factor II (*F2*), P-selectin (*SELP*), prolylcarboxypeptidase (*PRCP*) (34), and neuropeptide Y (*NPY*) (35). In this study, an additional gene with gender difference has been analyzed.

Physiological events are generally not regulated by a single gene. It remains to be investigated how the *ADRA2A* association is regulated on a molecular basis in lipid and glucose homeostasis. It is also of interest to study the effects of *ADRA2A* variants on response to administration of $\alpha_2$-adrenoreceptor agonists and antagonists. Not only single but also interactive effects of adrenergic receptor polymorphisms seem to contribute to obesity-related phenotypes such as hyperinsulinemia, insulin resistance, and increased systolic blood pressure (36,37). It is also important to study other components of the adrenergic receptor pathways in relation to the pathogenesis of T2D and obesity. In addition, other polymorphisms and environmental factors will give a better insight into the susceptibility, progression and treatment of obesity, T2D, and related disorders.

Variants in the $\beta_3$- and $\beta_2$-adenergic receptors have been previously associated with obesity and diabetes in several studies (38,39). There is a major interest in the possibility of receptor variants and their association with altered function and etiology of disease. Many candidate genes have been proposed for obesity and yielded conflicting associations with obesity-related traits (40). The reason for this might be sample size, different selected populations, gene–gene, and gene–environment interactions or lack of reproducibility.

## Conclusions

In summary, this study suggests that *ADRA2A* polymorphisms are associated with obesity and may also relate to T2D in a Swedish population. Further studies are needed to elaborate more on the function of *ADRA2A* and its possible preventive and therapeutic applications. O

## Acknowledgment

The authors thank Agneta Hilding for valuable statistical analysis discussion and Elisabeth Norén-Krog for excellent laboratory assistance.



## References

1. Bratusch-Marrain PR. Insulin-counteracting hormones: their impact on glucose metabolism. *Diabetologia* 1983;24:74–79.
2. Efendić S. Catecholamines and metabolism of human adipose tissue. 3. Comparison between the regulation of lipolysis in omental and subcutaneous adipose tissue. *Acta Med Scand* 1970;187:477–483.
3. Stich V, Pelikanova T, Wohl P, et al. Activation of alpha2-adrenergic receptors blunts epinephrine-induced lipolysis in subcutaneous adipose tissue during a hyperinsulinemic euglycemic clamp in men. *Am J Physiol Endocrinol Metab* 2003;285: E599–E607.
4. Lambert GW, Straznicky NE, Lambert EA, Dixon JB, Schlaich MP. Sympathetic nervous activation in obesity and the metabolic syndrome—causes, consequences and therapeutic implications. *Pharmacol Ther* 2010;126:159–172.
5. Clement K, Boutin P, Froguel P. Genetics of obesity. *Am J Pharmacogenomics* 2002;2:177–187.
6. Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. *Ann N Y Acad Sci* 2006;1083:129–152.
7. Ostenson C, Pigon J, Doxey J, Efendic S. Alpha 2-adrenoceptor blockade does not enhance glucose-induced insulin release in normal subjects or patients with noninsulin-dependent diabetes. *J Clin Endocrinol Metab* 1988;67:1054–1059.
8. Devedjian J, Pujol A, Cayla C, et al. Transgenic mice overexpressing alpha2A-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis. *Diabetologia* 2000;43:899–906.
9. Savontaus E, Fagerholm V, Rahkonen O, Scheinin M. Reduced blood glucose levels, increased insulin levels and improved glucose tolerance in alpha2A-adrenoceptor knockout mice. *Eur J Pharmacol* 2008;578:359–364.
10. Lima J, Feng H, Duckworth L, et al. Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations. *Metabolism* 2007;56: 757–765.
11. Ukkola O, Pérusse L, Chagnon Y, Després J, Bouchard C. Interactions among the glucocorticoid receptor, lipoprotein lipase and adrenergic receptor genes and abdominal fat in the Québec Family Study. *Int J Obes Relat Metab Disord* 2001;25: 1332–1339.
12. Rosmond R, Bouchard C, Björntorp P. A C-1291G polymorphism in the alpha2A-adrenergic receptor gene (ADRA2A) promoter is associated with cortisol escape from dexamethasone and elevated glucose levels. *J Intern Med* 2002;251:252–257.
13. Li J, Canham R, Vongpatanasin W, Leonard D, Auchus R, Victor R. Do allelic variants in alpha2A and alpha2C adrenergic receptors predispose to hypertension in blacks? *Hypertension* 2006;47:1140–1146.
14. Flordellis C, Manolis A, Scheinin M, Paris H. Clinical and pharmacological significance of alpha2-adrenoceptor polymorphisms in cardiovascular diseases. *Int J Cardiol* 2004;97:367–372.
15. Rosengren A, Jokubka R, Tojjar D, et al. Overexpression of $\alpha_{2A}$-adrenergic receptors contributes to Type 2 Diabetes. *Science* 2010;327:217–220.
16. Eriksson AK, Ekbom A, Granath F, Hilding A, Efendic S, Ostenson CG. Psychological distress and risk of pre-diabetes and Type 2 diabetes in a prospective study of Swedish middle-aged men and women. *Diab Med* 2008;7:834–842.
17. Alberti KGMM, Zimmet P. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diab Med* 1998;15:539–553.
18. National Heart LaBI. *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*. June 1998.
19. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* 1988;37:413–420.
20. A haplotype map of the human genome. *Nature* 2005;437:1299–1320.
21. Weir BS. *Disequilibrium. Genetic Data Analysis II: Methods for Discrete Population Genetic Data*, 2nd. ed. Sunderland, Massachusetts: Sinauer Associates; 1996, pp 91–140.
22. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19:149–150.
23. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419.
24. Wolfarth B, Rivera M, Oppert J, et al. A polymorphism in the alpha2a-adrenoceptor gene and endurance athlete status. *Med Sci Sports Exerc* 2000;32:1709–1712.
25. Freeman K, Farrow S, Schmaier A, Freedman R, Schork T, Lockette W. Genetic polymorphism of the alpha 2-adrenergic receptor is associated with increased platelet aggregation, baroreceptor sensitivity, and salt excretion in normotensive humans. *Am J Hypertens* 1995;8:863–869.
26. Michel M, Plogmann C, Philipp T, Brodde O. Functional correlates of alpha(2A)-adrenoceptor gene polymorphism in the HANE study. *Nephrol Dial Transplant* 1999;14:2657–2663.
27. Belfer I, Buzas B, Hipp H, et al. Haplotype-based analysis of alpha 2A, 2B, and 2C adrenergic receptor genes captures information on common functional loci at each gene. *J Hum Genet* 2005;50:12–20.
28. Kurnik D, Muszkat M, Li C, et al. Variations in the alpha2A-adrenergic receptor gene and their functional effects. *Clin Pharmacol Ther* 2006;79:173–185.
29. Brodde O, Bock K. Changes in platelet alpha 2-adrenoceptors in human phaeochromocytoma. *Eur J Clin Pharmacol* 1984;26:265–267.
30. Michel M, Mindermann G, Daul A, Brodde O. Effects of antihypertensive therapy on human alpha- and beta-adrenoceptors. *J Hypertens* 1991;9:601–606.
31. Luthra A, Borkowski K, Carruthers S. Genetic aspects of variability in superficial vein responsiveness to norepinephrine. *Clin Pharmacol Ther* 1991;49:355–361.
32. Långberg E, Gu HF, Nordman S, Efendic S, Ostenson CG. Genetic variation in receptor protein tyrosine phosphatase sigma is associated with type 2 diabetes in Swedish Caucasians. *Eur J Endocrinol* 2007;157:459–464.
33. O'Donnell C, Lindpaintner K, Larson M, et al. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 1998; 97:1766–1772.
34. McCarthy J, Meyer J, Moliterno D, Newby L, Rogers W, Topol E. Evidence for substantial effect modification by gender in a large-scale genetic association study of the metabolic syndrome among coronary heart disease patients. *Hum Genet* 2003;114:87–98.
35. Nordman S, Ding B, Ostenson C, et al. Leu7Pro polymorphism in the neuropeptide Y (NPY) gene is associated with impaired glucose tolerance and type 2 diabetes in Swedish men. *Exp Clin Endocrinol Diab* 2005;113:282–287.
36. Dionne I, Turner A, Tchernof A, et al. Identification of an interactive effect of beta3- and alpha2b-adrenoceptor gene polymorphisms on fat mass in Caucasian women. *Diabetes* 2001;50:91–95.
37. Ukkola O, Rankinen T, Weisnagel S, et al. Interactions among the alpha2-, beta2-, and beta3-adrenergic receptor genes and obesity-related phenotypes in the Quebec Family Study. *Metabolism* 2000;49:1063–1070.
38. Rosmond R. Association studies of genetic polymorphisms in central obesity: a critical review. *Int J Obes Relat Metab Disord* 2003;27:1141–1151.
39. Leineweber K, Büscher R, Bruck H, Brodde O: Beta-adrenoceptor polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* 2004;369:1–22.
40. Rankinen T, Zuberi A, Chagnon Y, et al. The human obesity gene map: the 2005 update. *Obesity* 2006;14:529–644.