www.sciencemag.org/cgi/content/full/1176827/DC1

Supporting Online Material for

Overexpression of Alpha2A-Adrenergic Receptors Contributes to Type 2 Diabetes

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Published 19 November 2009 on *Science* Express
DOI: 10.1126/science.1176827

This PDF file includes:

I. MATERIALS AND METHODS

Animals. The non-diabetic inbred F344/DuCrl2Swe rat was originally purchased from Charles River Laboratories (Wilmington, MA) and diabetic GK/Swe rats were originally from Kyoto University. Transfer of GK alleles onto the genome of F344 rats by repeated backcrossing for 10 generations established the homozygous congenic strain F344.GK-*Niddm1i* (NIDDM1I) with mitochondrial DNA and sex chromosomes from F344 (S*1*). NIDDM1I contains 0.6% of the GK genotype (16 Mb) on the genetic background of homozygous F344. The congenic strains N1I5, N1I11 and N1I12 were generated from an F2-intercross between F344 and NIDDM1I where animals with critical recombinations were selected for sister-brother breeding (S*2*). No major sex-specific differences have been observed in previous characterizations of *Niddm1* (S*3*); however, only males were included in this study to avoid possible effects of the estrus cycle. Animals used for experiments were 60 to 100 days old. F344 rats and the congenic NIDDM1F strain contain F344 genotype in the entire *Niddm1i* region and exhibit the same insulin secretion phenotype (S*2*,*S3*). NIDDM1F was used as control in the electrophysiological experiments and F344 in all other experiments. Rats were kept at constant temperature and humidity in a 12-h cycle of light and dark with free access to standard laboratory chow and water. Animal husbandry and the methods for the in vivo measurements and sacrificing the rats were approved by the local Ethics Committees.

Rat genotyping. Genomic DNA was extracted from tail biopsies. Genetic markers were selected from public databases or in-house information and were mapped within *Niddm1i* (S*3*). The location of markers was determined from Ensembl Rattus Norvegicus version 33.34c based on RGSC 3.4 (http://www.ensembl.org). The markers used for mapping of N1I11 and N1I5 are shown in Table S3. PCR amplification was conducted with one primer in each pair labeled with hex or fam fluorescence tags (DNA technology A/S, Denmark) using the primers listed in Table S3. The PCR products were separated on an ABI3100 (Applied Biosystems).

Intraperitoneal glucose tolerance tests (IPGTT). IPGTTs were performed in rats after 6 h fasting without anesthesia. Glucose was dissolved in 0.9% NaCl and 1.0 g glucose/kg body weight was delivered by intraperitoneal injection. Serial blood sampling by the retrobulbar approach at 0, 5, 15, 30, 90, and 180 min of the IPGTT was performed as previously described (S*4*). Blood glucose was analyzed using an AccuChek blood glucose reader. Plasma insulin was analyzed by radioimmunoassay. Clonidine or yohimbine were injected as indicated at 50 nmol/kg body weight and 2.5 μmol/kg body weight, respectively, 20 min prior to the IPGTT, and they were also dissolved in the IPGTT glucose solution at the same concentrations to ensure their presence during the tolerance test. Total volume load was 0.5-1 ml. The k-value was calculated by the standard formula k = 100 * (ln 2) / half time of glucose decline using the log values of the glucose concentrations from 5 to 180 min for estimation of the half time.

Islet isolation and in vitro insulin release from rat and human islets. Isolated pancreatic islets were prepared by collagenase digestion, hand-picked and incubated in a humidified atmosphere in RPMI 1640 tissue culture medium (SVA) supplemented with 10 % (vol/vol) fetal calf serum, 100 i.u. ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Insulin secretion in vitro was measured in static incubations. Prior to experiments, islets were preincubated for 30 min at 37 °C in a Krebs-Ringer bicarbonate buffer composed of 120 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM

$MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO, 2.8 mM glucose and 10 mM HEPES (pH 7.4) with 0.1% bovine serum albumin. The medium was gassed with 95% O_2 and 5% CO_2 to obtain constant pH and oxygenation. Next, groups of 10 islets (12 for human experiments) were incubated in 1 ml for 30 or 60 minutes, depending on the experimental conditions, at 37 °C in Krebs-Ringer buffered solution supplemented with glucose, clonidine, yohimbine, BRL-44408 (2-((4,5-dihydro-1H-imidazol-2-yl)methyl)-2,3-dihydro-1-methyl-1H-isoindole), deltamethrin, perimethrin or FK506 as indicated. Immediately after incubation, a 25 µl aliquot of the medium was removed for analysis of insulin content by radioimmunoassay. For pertussis toxin experiments, islets were incubated overnight in the absence or presence of the toxin (100 ng ml^{-1}). In the experiments testing the effects of deltamethrin, perimethrin and FK506 islets were preincubated with the compounds for 30 min prior to experiment as well as during the static batch incubations. FK506 was from CFM Oskar Tropitzsch, Germany. All other reagents were from Sigma-Aldrich, Sweden. Secretion experiments with human islets were performed according to the same protocol. All islet donors had given consent to donate organs for medical research. All procedures on human islets were approved by the ethical committees at Uppsala and Lund Universities.

Islet cell preparation and electrophysiology. Single β-cells were prepared by trituration in a Ca^{2+}-free medium. The resultant cell suspension was plated on plastic Nunc 35-mm Petri dishes and maintained in culture medium for up to 24 h. The electrophysiological measurements were conducted using an EPC-10 patch clamp amplifier with the PULSE software (version 8.64; HEKA). The plastic Petri dishes were used as the experimental chamber with a plastic insert to reduce the volume to approximately 0.5 ml. The dish was continuously perfused at a rate of ~2 ml min^{-1} at 31-33°C. Patch pipettes were pulled from borosilicate glass, coated with Sylgard and fire-polished to an average resistance of 4-6 MΩ when filled with pipette solution. The zero-current potential of the pipette was adjusted with the pipette in the bath. Exocytosis was monitored as increases in cell capacitance using the sine + DC mode of the lock-in amplifier included in the PULSE software and the standard whole-cell configuration. β-cells were identified based on their size and the inactivation properties of the voltage-gated Na^+ currents (S5). The extracellular solution in Fig. 1D consisted of (mM) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5.0 HEPES, and 5.0 glucose (pH 7.4 with NaOH). In Figs. 1C, 2D, S2D and S9, 20 mM TEACl substituted for equimolar amounts of NaCl to block outward K^+ channels that otherwise obscured the (smaller) inward Ca^{2+} currents. The pipette solution contained in Fig. 1D (mM) 125 K-glutamate, 10 KCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, 3 ATP, 0.1 cAMP, 10 EGTA and 9 $CaCl_2$ (pH 7.2 with KOH). In the remaining figures the intracellular solution consisted of (mM) 125 Cs-glutamate, 10 CsCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, 3 ATP, and 0.1 cAMP and 0.05 EGTA, (pH 7.2 with CsOH). All reagents were from Sigma-Aldrich. For pertussis toxin experiments, islets incubated with pertussis toxin as described above were dispersed into single cells and 100 ng ml^{-1} of the toxin was added also to the single-cell suspension to allow for continuous presence of pertussis toxin until experiments were performed.

$[Ca^{2+}]_i$ measurements. $[Ca^{2+}]_i$ was estimated by dual-wavelength microfluorimetry (S6). The islets were loaded with 3 µM fura-2 in the presence of 0.007% pluronic acid (Invitrogen, Sweden) for 40 minutes at 37 ºC prior to the experiments. The islets were washed and transferred to a glass-bottomed dish in a solution containing (mM) 140 NaCl, 3.6 KCl, 2 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgSO_4$, 5 HEPES, 2.5 $CaCl_2$ and 5 glucose (pH 7.4 with NaOH). Upon

stimulation, glucose or K^+ were added to final concentrations of 20 and 60 mM, respectively. In the solution used for stimulating the islets by elevating [KCl] to 60 mM, the amount of NaCl was reduced correspondingly to maintain constant osmolarity. In the clonidine and yohimbine experiments, clonidine or yohimbine were added to the extracellular solution to final concentrations of 1 and 10 μM, respectively, approximately 10 minutes after the glucose stimulation. The islets were analyzed using a NIKON Diaphot microscope with a heated microscope stage (32-34 ºC), connected to a Photon Technology International (PTI, N.J., USA) imaging system in an optical plane close to the lower surface of the islets. Fura-2 was excited alternately at 340 and 380 nm and fura-2 fluorescence was measured continuously. The fluorescence intensity ratios (F_{340}/F_{380} nm) were calculated and analyzed using the PTI Image Master Software. The ratios were normalized to the maximum ratio, achieved using 60 μM ionomycin.

Electron microscopy. Freshly isolated rat islets were incubated in the standard medium (with 5.5 mM glucose) prior to fixation. Human islets used for electron microscopy were preincubated in 2.8 mM glucose for 30 min followed by 60 min incubation at 20 mM glucose with or without alpha(2A)AR antagonist (according to the procedure outlined in Islet isolation and in vitro insulin release). After the incubation period, islets were fixed in 2.5% glutaraldehyde for 1 h, treated with 1% osmium tetroxide, dehydrated and embedded in Durcupan. β-cells were readily identified by the typical appearance of the granules with a central dense core and a surrounding halo, and only these cells were used for the analysis. Cellular granule distribution was determined using in-house MATLAB-based software. A granule was considered as docked if the granule centre was within 0.2 μm, i.e. half a granule diameter, from the plasma membrane.

RNA isolation and expression analysis. For rat islet RNA extraction, islets were homogenized in TRIzol reagent (Invitrogen Life Technologies) immediately after collagenase digestion of the pancreas. RNA was isolated from islets (n=3-6 rats/strain) by extraction with chloroform followed by isopropanol precipitation. Samples with a 260/280 nm ratio above 1.8 were selected for cDNA synthesis using Omniscript Reverse transcription (Qiagen) with addition of RiboLock™ Ribonuclease inhibitor (Fermentas GmbH, Germany). The oligonucleotides used for quantitative real-time PCR are listed in Table S3. Oligonucleotides for *Adra2a* and *Pdcd4* were assays ordered on-demand (Applied Biosystems). Gene expression was assessed in ABI PRISM 7900 (Applied Biosystems) with a reaction volume of 10 μl. Samples were analyzed in duplicates under the following conditions: 50°C for 2 minutes, 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The *Adra2a* transcript was significantly different between the strains at 25 cycles. The transcript quantity was normalized to the mRNA level of *Hprt*.

RNA isolation from human islets was performed using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA) and an ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA). Concentration and purity was measured using a NanoDrop ND-1000 spectrophotometer (A260/A280>1.8 and A260/A230>1.0) (NanoDrop Technologies, Wilmington, DE, USA). No sign of degradation was observed using agarose gel electrophoresis and Experion DNA 1K gel chips (Bio-Rad, Hercules, CA, USA). Reverse transcription was performed using 1 μmol/l dT18 oligomer and 3 μmol/l random hexamer primers. Quantitative real-time PCR was carried out using an ABI 7900HT sequence detection system with 10 ng cDNA in 10 μl reaction volumes and TaqMan Expression PCR Master Mix with duplex assays

according to the manufacturer's recommendation (Applied Biosystems). Triplicates were run, and the *ADRA2A* transcript quantity was normalized to the mRNA levels of *HPRT1* and *Cyclophilin A*. Assays used were: *ADRA2A* Hs00265081_s1, *HPRT1* (4326321E, VIC-MGB) and *Cyclophilin A* (4326316E, VIC-MGB).

Western blot. The tissue expression pattern of alpha2-adrenergic receptor subtypes is not fully known but both brain and pancreatic islet tissue have been demonstrated to express alpha(2A)AR (S*7, S8*). Brain tissue was collected from congenic rats and quickly frozen in liquid nitrogen and stored in -80°C until homogenization. The homogenization buffer consisted of (mM): 50 TRIS, 5 $MgCl_2$, 1 $CaCl_2$, 1 EDTA, 0,1 μM EGTA, 0,5% Triton X-100 (pH 7.4) and complete protease inhibitor cocktail (Roche). After homogenization, debris was removed by centrifugation and the supernatants were collected. For islet experiments, freshly isolated rat islets (or donated human islets) were transferred to the homogenization buffer (1000 islets to 250 μl buffer), sonicated and frozen before analysis. Protein concentration was determined according to Bradford (1976). Fifty micrograms of total protein content was electrophoresed on 10% SDS-PAGE, and the separated proteins were transferred onto a polyvinylidine fluoride membrane (Amersham Pharmacia Biotech). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline with Tween (0.15 mM NaCl, 10 mM Tris-HCl and 0.05% Tween20; pH 7.4) for 1 h at room temperature. After blocking, the membrane was incubated with polyclonal rabbit anti-alpha(2A)AR antibody (1:250) (Sigma-Aldrich) and monoclonal rabbit anti-β-actin antibody (1:500) in Tris-buffered saline with Tween for 1 h at room temperature. After washing, the membrane was incubated with peroxidase-linked anti-rabbit IgG (1:25000) (Amersham) overnight at 4°C. Signal was detected by a chemiluminescence kit (Thermo Scientific). Western blots were scanned using a FujiFilm LAS300 Intelligent dark box scanner, and the band density of the blots was quantified within the linear range of detection with the LAS Image Reader software and analyzed with the MultiGauge software (FujiFilm). Only the mature glycosylated 70 kDa band that has previously been demonstrated as the major band of alpha(2A)AR in immunoblots of brain tissue using this antibody was detected (S*9, S10*). The alpha(2A)AR signal was normalized for β-actin.

Promoter activity assay. To assess if the effect of the *Adra2a* promoter (S*11*) on gene expression depended on having GK or F344 genotype in the promoter region we constructed the recombinant plasmids pGL4.10[*luc2*]F344 and pGL4.10[*luc2*]GK, both expressing the *Firefly* luciferase. A 1934 bp genomic fragment from GK or F344 encompassing the 2 SNPs in the 5' UTR of *Adra2a* was amplified in a total reaction volume of 10 μl containing 10 ng genomic DNA, 1.6 mM $MgCl_2$, 0.15 mM of each dNTP, 1 μM of each primer, 0.5 M Betaine (Sigma-Aldrich) and 0.04 U Taq Polymerase (MBI Fermentas). The PCR amplification was performed in a GeneAmp PCR System2700 (Applied Biosystem) with an initial denaturation step of 4 min at 94 °C followed by 35 cycles with 40 s at 94°C, 30 s at 60°C, and 90 s at 72°C. A final elongation step (7 min at 72°C) ended the reaction. DNA fragments were separated and visualized by electrophoresis on 0.8 % agarose gels containing ethidium bromide, and the fragments were gel-purified using the Qiagen Gel Extraction kit. The following oligonucletotides were designed using the software OligoPerfect™ Designer (Invitrogen):
5'-AGTC-GGTACC-CCAAGCTCCTACCATTCTCATC-3' (forward) and
5'-AGTC-CTCGAG-CGGGGTCCTAGGTCTGTCTT-3' (reverse)

on the basis of the nucleotide sequence deposited in GenBank, entry NW_001084775.1. The forward and reverse oligonucleotide primers contained restriction sites for Acc65I and XhoI (underlined). The purified fragments were cleaved by Acc65I and XhoI and ligated into the pGL4.10[*luc2*] vector using T4 DNA ligase (MBI Fermentas) according to the manufacturer's recommendation. Each plasmid was transformed into *E. coli* XL10-Gold ultracompetent cells (Stratagene) according to the manufacturer's specifications. Orientation of each construct was confirmed by *Xba*I restriction endonuclease digestion. The insert into selected clones was verified by bi-directional sequencing using the BigDye terminator kit (Applied Biosystems). The rat-derived insulin-secreting RINm5F cell line (passages 80 to 95) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 i.u. ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37°C. RINm5F cells were transiently transfected with the *Firefly* luciferase-expressing vectors using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. To enable estimation of transfection efficiency, cells were co-transfected with a vector expressing the *Renilla* luciferase constitutively (pGL4.74[*hRluc*/TK]). Cells were harvested 24 hours after transfection in a Passive Lysis Buffer (Promega). Cell debris was removed after centrifugation and the supernatant was assayed for luciferase activity with the Dual Luciferase Assay system (Promega) using a MiniLumat LB 9506 (Berthold Technologies). The level of expression represents the ratio of *Firefly* and *Renilla* luciferase activity. The pGL4.10[*luc2*] vector without the *Adra2a* promoter gene fragment was used as negative control.

RNA interference. Freshly isolated rat islets were incubated in the standard culture medium as specified above for 1 hour prior to transfection. Next, 60-80 islets were transferred to 1.7 mL RPMI 1640 tissue culture medium supplemented with 10 % (vol/vol) fetal calf serum but without antibiotics. To 300 mL Opti-Mem medium (without fetal calf serum) was added 7 μl lipofectamine RNAiMAX, 4.5 μl BLOCK-It Alexa Fluor Red Fluorescent Oligo, and totally 4.5 μl of siRNA oligonucleotides. To decrease the risk of off-target RNA interference, 1.5 μl of each of three different siRNA oligonucleotides targeting *Adra2a* were used concomitantly, thereby allowing the concentration of each construct to be reduced. For control experiments, 4.5 μl of Stealth RNAi Negative Control with 55–65 % GC content was used. After 20 min incubation at room temperature, this cocktail was added to the islet suspension, giving a total volume of 2 ml and a final siRNA concentration of 45 nM. Following 12 h incubation at 37°C, the medium was changed to the standard culture medium with antibiotics. The cell medium was thereafter changed daily until experiments were performed, 48-60 h post-transfection. All reagents were from Invitrogen, and the *Adra2a* siRNA oligonucleotides were ordered as assays on-demand (Invitrogen). The BLOCK-It Alexa Fluor Red Fluorescent Oligo was added to allow for visualization of transfected cells. As the Alexa Fluor Red-coupled oligonuclotides are bulkier than the *Adra2a* siRNA oligonucleotides, this approach is unlikely to overestimate the transfection rate. Transfection efficiency was also assessed by Western blot (Fig. 2B) and functional experiments (Fig. 2C). Islet fluorescence was visualized using a Zeiss LSM 510 META system with an inverted Zeiss Axiovert 100 M microscope and LSM 510 META software version 3.2 (Carl Zeiss). The Alexa Fluor Red Oligo was excited with the 543 nm line of a helium-neon laser and emission was collected via a Zeiss Plan-Apochromat 10x objective using a band-pass 560-615 nm filter. For confocal images (Fig. S8), the pin-hole was adjusted to 1 Airy unit. For measurements of exocytosis from *Adra2a*-silenced β-cells, transfected islets were dispersed into single cells as described above. Only cells that showed clear Alexa fluorescence were selected for capacitance recordings at an adjoined patch-clamp setup.

cAMP measurements. Islets were preincubated for 30 min at 37° in a Krebs-Ringer bicarbonate buffer (see the Islet isolation and in vitro insulin release section). The medium was gassed with 95% O_2 and 5% CO_2 to obtain constant pH and oxygenation. Next, groups of 25 islets were incubated in 1 ml for 5 or 30 minutes at 37 °C in Krebs-Ringer buffered solution supplemented with 2.8 or 20 mM glucose with or without clonidine or yohimbine. 100 μM isobutylmethylxanthine (IBMX) was present in the incubation medium (but not during preincubation). Immediately after incubation, the islets were transferred (in a total volume of 25 μl Krebs-Ringer solution) to 200 μl 0.1 M HCl supplemented with 100 μM IBMX. After 15 minutes in room temperature, the suspension was frozen overnight. Concentration of cAMP was assayed by Cyclic AMP EIA Kit (Cayman Chemical Company, Michigan, USA) with no acetylation according to the manufacturer's protocol.

Study population. The Botnia study was initiated in 1990 and is a family-based study aiming to identify genes that increase T2D susceptibility (S*12*). From the Botnia study, a sample of 935 individuals well-characterized for insulin secretion through IVGTT was genotyped for analysis of 19 SNPs. 136 subjects with T2D were excluded from analysis. During IVGTT, plasma glucose and insulin concentrations were measured at 0, 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 min. Five SNPs were selected for replication based on their association with impaired insulin secretion in the Botnia IVGTT cohort as well as their genomic position, the latter criterion in order to capture a large chromosomal region also in the replication study. The five SNPs were replicated in another cohort of the Botnia study, the Prevalence, Prediction and Prevention of T2D (Botnia PPP) study, including 4935 individuals aged 18–74 years. 254 individuals with T2D were excluded from analysis. These individuals underwent an OGTT, and blood samples were drawn at 0, 30 and 120 min of the OGTT. Plasma glucose was measured with the glucose oxidase method and plasma insulin concentrations were measured with ELISA (Dako). Diagnosis of diabetes was confirmed from subject records or on the basis of a fasting plasma glucose concentration above 7.0 mmol/l and/or 2 h glucose above 11.1 mmol/l. For statistical analyses, we used the corrected insulin measures, calculated according to the formula:

$$CorrectedInsulin_T = 100 \times Insulin_T / (Glucose_T \times (Glucose_T - 3.89))$$

where glucose is in mmol/l, insulin in mU/l and T denotes the time point of measurement. HOMA-IR was calculated as fasting glucose (mmol/l) x fasting insulin (mU/l) / 22.5. Insulin sensitivity index (ISI) was calculated from the OGTT as $10000/\sqrt{((\text{fasting glucose} \times \text{fasting insulin})(\text{mean OGTT}_{\text{glucose}} \times \text{mean OGTT}_{\text{insulin}}))}$. Diabetics were removed from the phenotype analyses.

The case-control sample included 2830 Scandinavian patients with T2D (from the local Diabetes Registry in Malmö(S*13*)) and 3740 unrelated ethnically matched healthy control persons (from the Malmö Diet and Cancer Study(S*14*)). T2D was diagnosed according to the WHO criteria with C-peptide concentrations ≥0.3 nmol/l, no GAD antibodies and age at onset above 35 but below 75 years of age. The control individuals had fasting blood glucose <5.5 mmol/l, HbA_{1c} below 6%, and no known first-degree relative with diabetes. Subjects with type 1-diabetes or genetically verified MODY were excluded from both studies. All participants gave informed consent and the protocols were approved by the local ethics committees. There was no known overlapping of individuals between any of the cohorts used in this study. See also Table S2 for details on the different study samples.

Genotyping. 19 SNPs spanning 500 kb upstream and 500 kb downstream of *ADRA2A* were chosen from the International Hapmap Project (http://www.hapmap.org) using the program TAGGER (S*15*) with aggressive tagging, r^2>0.8 and LOD>3.0. Seven SNPs (rs7911129, rs7075340, rs1890920, rs2419566, rs946940, rs1971596, rs17128356) were selected from the region 500 kb upstream of *ADRA2A*, two SNPs were from the promoter region (rs521674, rs1800763), one from the 5' UTR region (rs1800545), three from the 3' UTR region (rs11195419, rs553668, rs13306146), and six SNPs (rs602618, rs4918621, rs4918625, rs1360866, rs7100191 and rs2203616) were selected from the 500 kb downstream region of the gene.

Nine SNPs (rs11195419, rs13306146, rs17128356, rs1800545, rs1800763, rs4918621, rs4918625, rs521674 and rs602618) were genotyped by primer extension of multiplex products with detection by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry using the iPLEX protocol on a Sequenom MassARRAY platform (Sequenom) at RSKC, UMAS, Malmo, Sweden. The remaining 10 SNPs were genotyped using an allelic discrimination assay-by-design method on ABI 7900 (Applied Biosystems). Native DNA was used for all genotyping. We obtained an average genotyping accuracy >99% as seen from re-genotyping random samples of 5%. All SNPs were in HWE in control individuals with P>0.05. Detailed SNP data are given in Table S1, and linkage disequilibrium between SNPs is shown in Figure S12.

Statistical analysis. For cellular and animal experiments, data are presented as mean values ± s.e.m. The unpaired two-tailed Student's *t*-test was used for all comparisons. For Western blot experiments, alpha(2A)AR expression normalized to β-actin expression was compared between the different cohorts (N1I5 vs. N1I11; inactive vs. active siRNA-treated islets; GG vs. GA/AA carriers) in each blot to obtain a measure of relative expression in that particular blot. The relative expression in 4-5 blots per experimental condition was analyzed by the unpaired two-tailed Student's *t*-test. In insulin secretion assays from human islets, 4-8 independent islet batches were analyzed per individual, and the average response for each individual was used for the genotype-based comparisons. The number of individuals included in the insulin secretion experiments (Fig. 3D) was for GG carriers: n=25 at 2.8 mM glucose, n=3 at 2.8 mM glucose + antagonist, n=12 at 20 mM glucose and n=6 at 20 mM + antagonist. For risk allele carriers (denoted "GA" in Fig. 3D): n=8 GA and 1 AA at 2.8 mM glucose, n=2 GA at 2 8 mM glucose + antagonist, n=6 GA at 20 mM glucose and n=6 GA at 20 mM glucose + antagonist). Islets from 4 GG and 3 GA carriers were analyzed by electron microscopy. Granule distribution in 12-28 cells per condition and genotype were examined as described in the electron microscopy section. To increase power in the human islet secretion and granule distribution analyses both yohimbine (alpha(2A/2B/2C)AR antagonist) and the specific alpha(2A)AR antagonist BRL44408 (S*16*) were used when testing the effects of alpha(2A)AR antagonism. It was ascertained that the effects of yohimbine and BRL44408 were identical (GG 20 mM glucose: yohimbine 1.40±0.22, BRL 1.34±0.16, and GA 20 mM glucose: yohimbine 1.49±0.31, BRL 1.40±0.12).

Linkage disequilibrium between SNPs was calculated using Haploview (ver. 4.1) and D' values were calculated with 95% CIs. Prevalence of T2D between different genotype carriers was analyzed by Fisher's exact test (1 df). Odds ratio (OR) was determined by binary logistic regression. The genotype-phenotype correlations were analyzed by ANOVA, or by linear

regression analyses in cases where an additive model could be applied. For the initial analysis of the 799 subjects, all 19 SNPs were analyzed with two-tailed comparisons. For the replication study, one-tailed comparisons were performed. Blood pressure and insulin sensitivity measures were analyzed by two-tailed comparisons. As insulin secretion was non-normally distributed, data were logarithmically transformed before analysis. The acute insulin response (AIR) to glucose was calculated from the +2- to 6-minute values of the IVGTT and analyzed by two-tailed comparisons. The data were adjusted for family history of diabetes, as well as age and sex differences. For blood pressure analyses, individuals with T2D or hypertensive medication were removed. All statistical analyses were performed using SPSS Statistics (ver 15.0; SPSS Inc).

II. SUPPORTING FIGURES

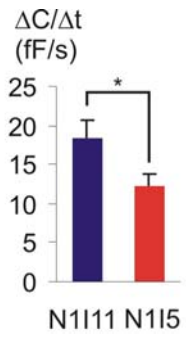

Figure S1 Ca^{2+}-evoked exocytosis.
ΔC evoked by intracellular infusion of a Ca^{2+}- (free $[Ca^{2+}]_i$ ~ 1.5 μM) containing solution after the establishment of the standard whole-cell configuration. The histogram presents the average exocytotic rates (ΔC/Δt) in N1I11 and N1I5 β-cells (n=7 for N1I11 and 11 for N1I5). * $P<0.05$.

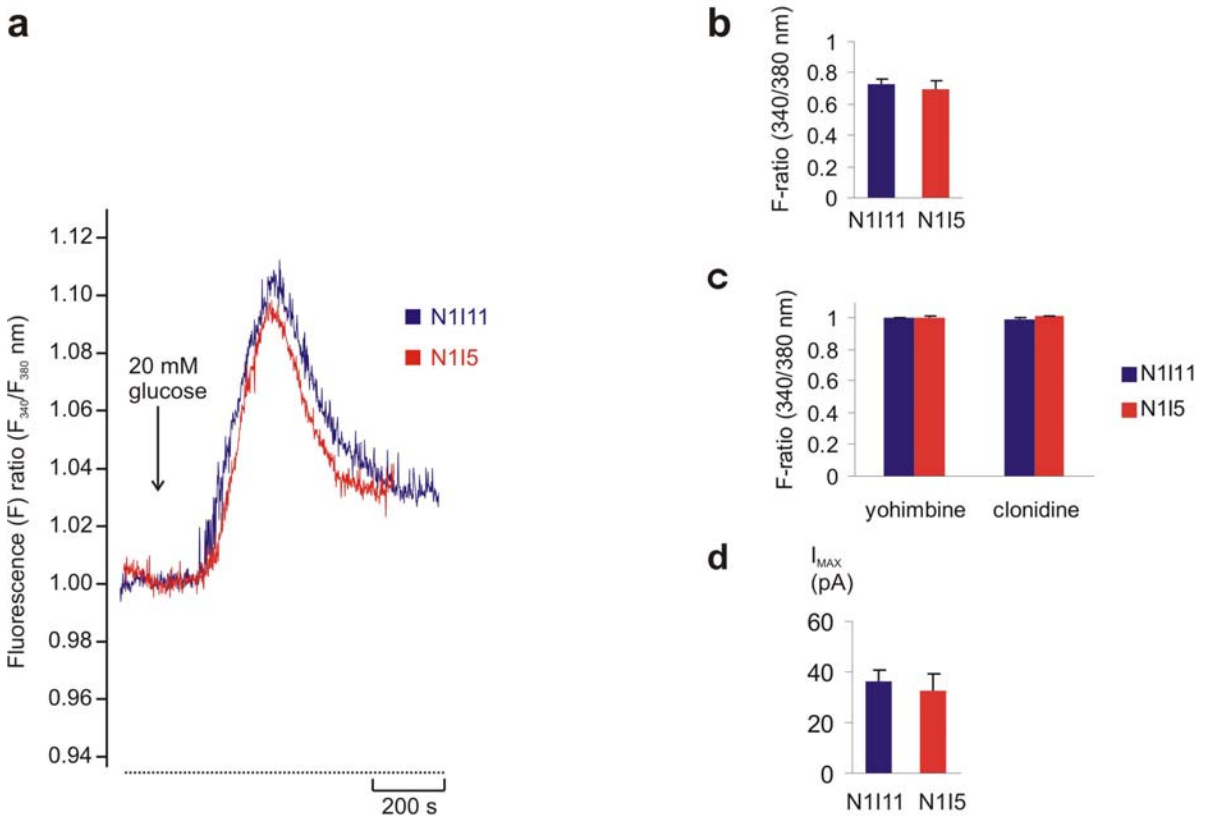

Figure S2 Ca^{2+} homeostasis in N1I11 and N1I5.
(**a**) Recordings of fura-2 fluorescence (F) ratio (F_{340}/F_{380}) in an N1I11 and an N1I5 islet. The arrow indicates the elevation of glucose concentration from 5 to 20 mM. (**b**) Base-line F-ratio at 5 mM glucose in N1I11 and N1I5 islets normalized to the maximum F-ratio achieved during the experiment. n=7-8 per strain. (**c**) Yohimbine or clonidine was added to some islets (n=4 per strain and compound) approximately 10 minutes after the elevation of glucose to 20 mM. Data are the average F-ratio following yohimbine or clonidine addition normalized to the F-ratio immediately before the inclusion of the compounds (i.e. the comparisons were made relative to the F-ratio 10 minutes post-glucose elevation). (**d**) Peak Ca^{2+} current (I_{MAX}) evoked by the first depolarization of the train stimulus in N1I11 and N1I5 β-cells during the capacitance measurements. (n= 8 for N1I11 and 11 for N1I5).

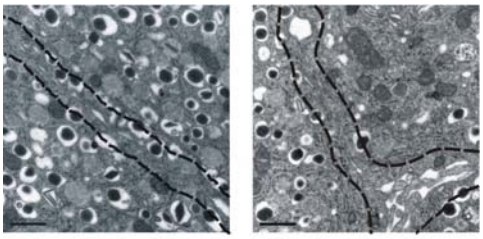

Figure S3 Ultrastructural analysis of congenic islets.
Electron micrographs of rat islet sections from N1I11 and N1I5 showing β-cells with insulin granules (black bar in lower left corners: 0.5 μm). Total number of granules was 270±15 and 250±13 per β-cell and section in N1I11 and N1I5, respectively. Dashed lines indicate a distance of 0.2 μm from the plasma membrane.

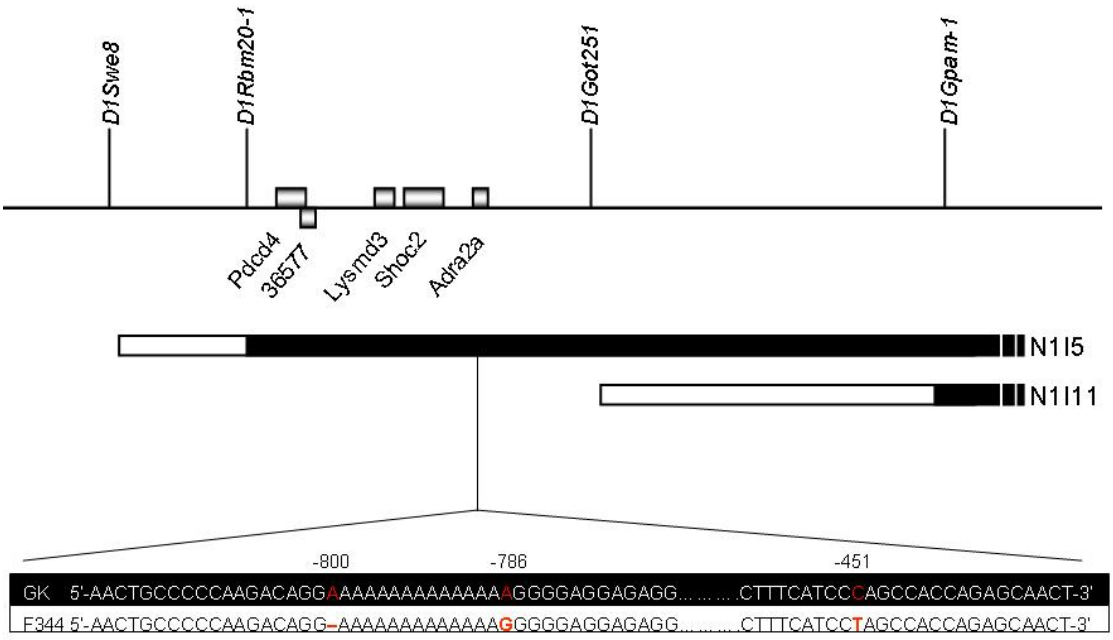

Figure S4 GK-derived genetic segments of congenic strains.
The filled bars illustrate known homozygous GK-derived genetic segments; the open ends show intervals containing the recombinant end points. The microsatellite markers used for genetic mapping of N1I5 and N1I11 as well as the known protein-coding genes in the interval between the strains are shown. 36577 designates the gene *ENSRNOG00000036577*. Shown are also sequence differences (red) between F344 and GK in the *Adra2a* promoter. There was one insertion/deletion at 800 bp upstream of the transcription initiation site (-800 bp; $F344^{-}/GK^{A}$) and two single nucleotide polymorphisms (SNPs) at -786 bp ($F344^{G}/GK^{A}$) and -451 bp ($F344^{T}/GK^{C}$). There were no sequence differences in the coding region or the nearest 1694 bp 3' UTR region of the gene.

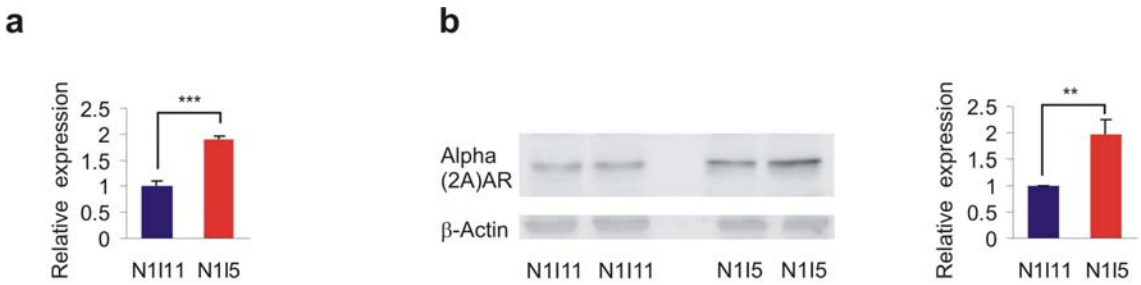

Figure S5 Expression of alpha(2A)AR in islets and brain from N1I11 and N1I5 rats.
(**a**) Average relative alpha(2A)AR signal normalized for β-actin in N1I5 vs. N1I11 islets in 4 blots from totally 12 rats/strain. (**b**) Immunoblots of total protein from 2 brain samples from each strain using polyclonal alpha(2A)AR antisera. The bars show average relative alpha(2A)AR signal normalized for β-actin in N1I5 vs. N1I11 brain tissue in 5 blots from totally 3 rats/strain. ** P<0.01, *** P<0.001.

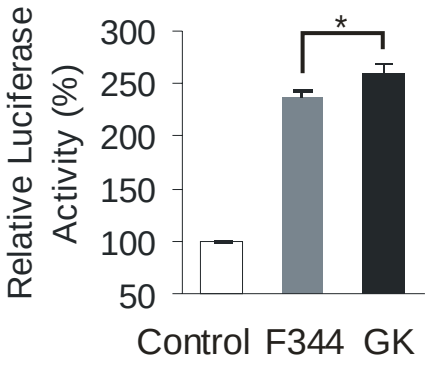

Figure S6 Effects of F344- or GK-derived *Adra2a* promoter.
To compare the effects of F344- and GK-derived *Adra2a* promoter on gene expression we constructed the recombinant plasmids pGL4.10[*luc2*]F344 and pGL4.10[*luc2*]GK, both expressing the *Firefly* luciferase. The rat-derived insulin-secreting RINm5F cell line was transiently transfected with the pGL4.10[*luc2*]F344 ("F344" in the figure) and pGL4.10[*luc2*]GK ("GK") plasmids. To enable estimation of transfection efficiency, cells were co-transfected with a vector expressing the *Renilla* luciferase constitutively (pGL4.74[*hRluc*/TK]). The level of expression represents the ratio of *Firefly* and *Renilla* luciferase activity. The pGL4.10[*luc2*] vector without the *Adra2a* promoter gene fragment was used as negative control ("Control"). In line with the expression analyses, plasmid constructs containing the GK variant of the *Adra2a* promoter significantly enhanced reporter gene expression (8.3%, $P<0.05$; n=14) compared to the F344 variant when transfected into insulin-secreting RINm5F cells. * $P<0.05$.

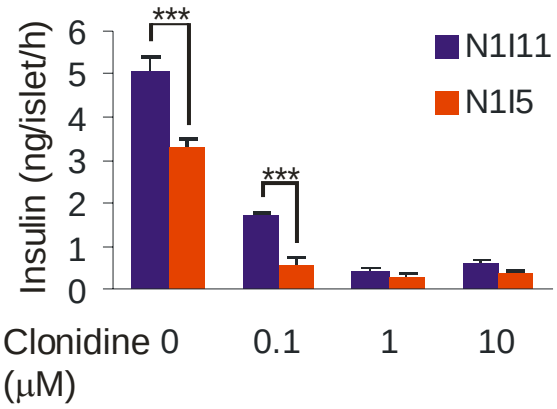

Figure S7 Effects of clonidine on insulin secretion in congenic islets.
Insulin secretion from N1I11 or N1I5 islets at 20 mM glucose with clonidine at different concentrations as indicated (n=8 per group). A maximal inhibitory effect of clonidine was achieved at 1 μM. *** P<0.001.

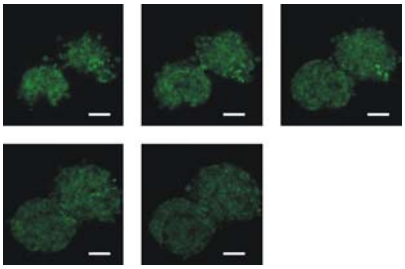

Figure S8 Visualisation of transfected islets.
Confocal images of Alexa-555 signal (green) from bottom (upper left) to top (lower right) at 10 μm intervals in an N1I5 islet transfected with active siRNA and BLOCK-It Alexa Fluor Red Fluorescent Oligo. Scale bar: 10 μm.

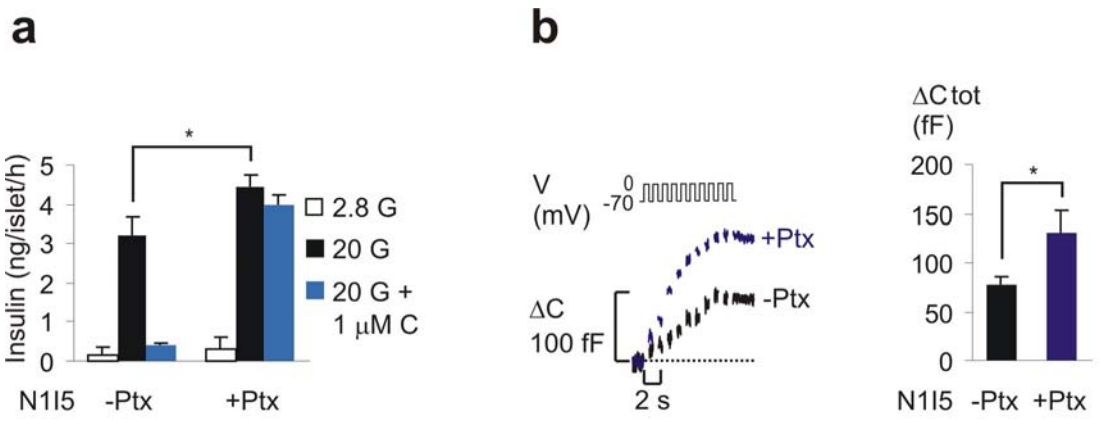

Figure S9 Effects of pertussis toxin treatment on insulin secretion and single β-cell exocytosis. (**a**) Insulin secretion from N1I5 islets without (-ptx) or with (+ptx) pertussis toxin pretreatment under conditions as specified (G=glucose in mM; C=clonidine; n=8 per group). (**b**) Exocytosis elicited by trains of ten depolarizations and measured as increases in cell capacitance (ΔC) in N1I5 β-cells in the absence or presence of pertussis toxin. The histogram shows total exocytosis evoked by the train stimulus (ΔC_{TOT}) in 6 cells per group. * $P<0.05$.

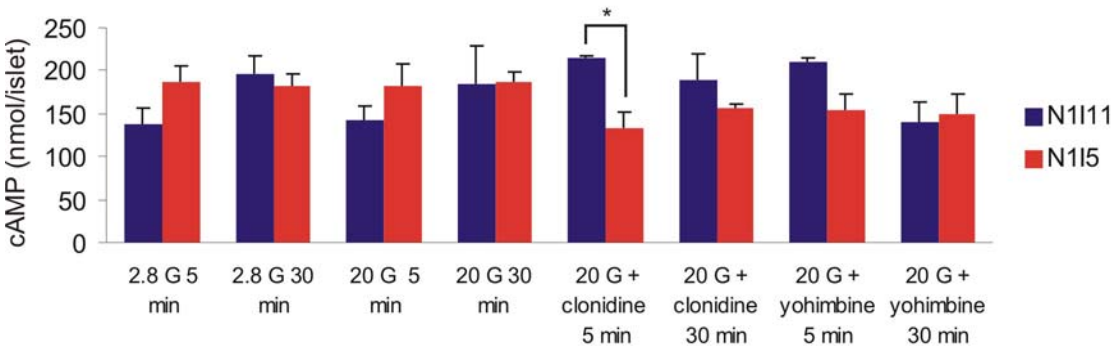

Figure S10 cAMP measurements in congenic islets.
The histogram shows cAMP levels in islets from N1I11 and N1I5 incubated for 5 or 30 minutes with 2.8 mM glucose ("2.8 G"), 20 mM glucose ("20 G"), 20 mM glucose + 1 μM clonidine ("20 G + clonidine"), or 20 mM glucose + 10 μM yohimbine ("20 G + yohimbine"). Isobutylmethylxanthine was present in the incubation medium. 25 islets were assayed in each well and n= 4-5 per group. Five rats per strain were used. * $P<0.05$.

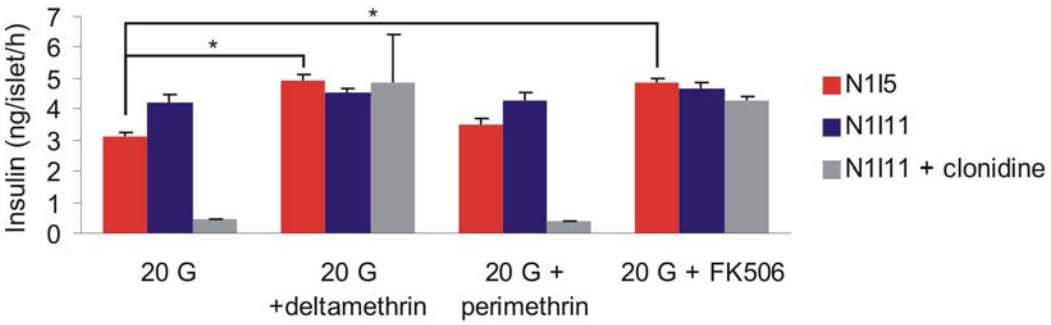

Figure S11 Effects of deltamethrin, perimethrin and FK506 on glucose-stimulated insulin secretion.

The histogram shows insulin secretion from N1I11 or N1I5 islets at 20 mM glucose (20 G) with 100 nM deltamethrin, 100 nM perimethrin or 100 nM FK506 as indicated. The compounds were present both in the preincubation medium for 30 minutes prior to experiment and during the 60-min stimulation. The effect of 1 μM clonidine on glucose-stimulated insulin release was also investigated for each compound. (n=3-8 per groups). * $P<0.05$.

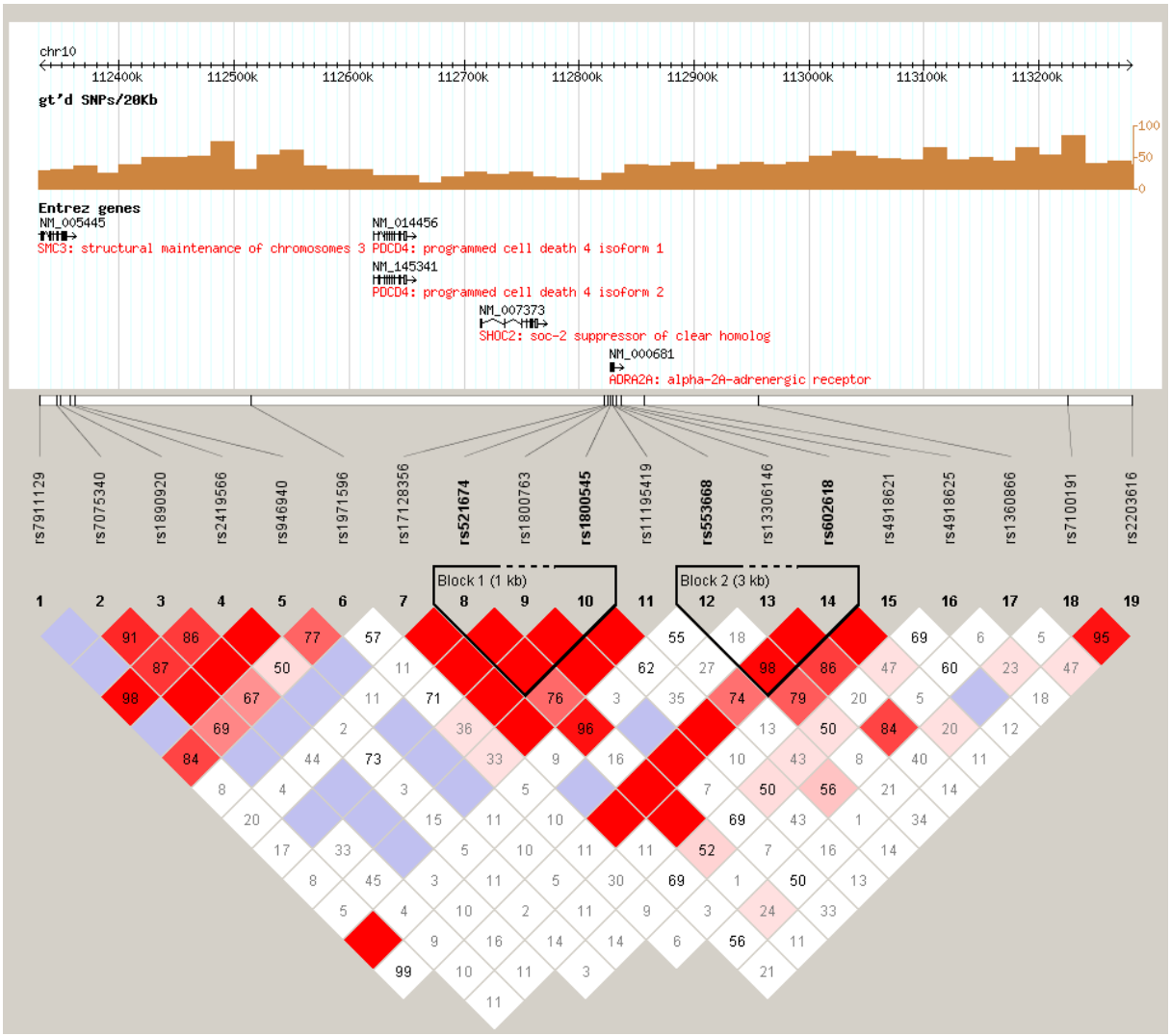

Figure S12 Linkage disequilibrium (LD) of the 19 selected SNPs across *ADRA2A* in the individuals included in the Botnia IVGTT study.
The positions of the SNPs are indicated. Yellow histogram depicts the number of HapMap-genotyped SNPs per 20Kb window across the chromosome. Genes in the region are indicated. The bottom part of the figure shows an LD plot based on the D' measurement. Each diamond represents the pairwise magnitude of LD, with red color indicating strong LD. The figure was prepared using Haploview, ver. 4.1 (Broad Institute).

SUPPORTING TABLES

Table S1 *ADRA2A* SNPs genotyped in the Botnia IVGTT cohort.
For each SNP is expressed the position on human chromosome 10, the percentage of genotyped individuals in the Botnia IVGTT cohort, minor allele frequency, and major:minor allele. SNPs in bold were used also in the replication study.

Name	Position	%Genotyped	MAF	Alleles
rs7911129	112330860	99.3	0.061	A:G
rs7075340	112346320	99.2	0.013	A:G
rs1890920	112349730	97.9	0.012	T:A
rs2419566	112358217	98.6	0.188	A:G
rs946940	112362000	99.8	0.012	C:T
rs1971596	112515074	99.4	0.439	C:G
rs17128356	112822215	99.9	0.0080	C:T
rs521674	112825580	98.6	0.247	A:T
rs1800763	112825982	93.2	0.0070	G:T
rs1800545	112827528	99.2	0.062	G:A
rs11195419	112829358	99.7	0.076	C:A
rs553668	112829570	97.3	0.143	G:A
rs13306146	112830161	100.0	0.017	A:G
rs602618	112833075	99.2	0.261	A:C
rs4918621	112837169	100.0	0.0080	C:A
rs4918625	112857209	99.8	0.485	T:C
rs1360866	112957070	98.8	0.391	T:A
rs7100191	113225874	99.2	0.462	T:C
rs2203616	113281290	99.6	0.18	C:A

Table S2 Effects of SNPs on P-glucose and S-insulin.
The effect of the 19 SNPs on fasting P-glucose (mmol/l) and S-insulin at 0, 30 and 60 min during an IVGTT for the Botnia IVGTT cohort, as well as the effect of the 5 SNPs that were replicated in the Botnia PPP cohort on P-glucose and S-insulin at 0, 30 and 120 min during an OGTT. Data are shown as means ± SD. Measures of insulin denote the corrected insulin response (mU x l/mmol^2). The genotype-phenotype correlation was analyzed by ANOVA, or by linear regression in case an additive model could be applied. Details on the case-control sample are also presented. For Botnia IVGTT and Botnia PPP individuals with T2D were excluded from analysis. NA not applicable.

		Genotypes			
Study	Phenotype	GG	GA	AA	P value
Botnia IVGTT (n=799)					
SNP					
rs7911129	Individuals	633	82	2	
	Age (y)	45±13	46±12	40±2	
	BMI (kg/m^2)	25.4±4.0	25.2±3.2	21.3±1.3	
	Fasting P-glucose	5.47±0.65	5.50±0.75	6.25±1.06	0.5
	Fasting S-insulin	6.19±3.54	6.46±4.33	3.07±1.82	0.9
	S-insulin 30 min	19.1±11.5	19.9±12.8	8.5±2.5	0.9
	S-insulin 60 min	16.0±12.1	16.1±14.3	8.0±5.6	0.07
rs7075340	Individuals	698	17	1	
	Age (y)	45±13	45±14	NA	
	BMI (kg/m^2)	25.4±3.9	26.0±4.1	NA	
	Fasting P-glucose	5.47±0.66	5.51±0.68	NA	0.5
	Fasting S-insulin	6.16±3.62	7.52±4.08	NA	0.3
	S-insulin 30 min	19.2±3.6	18.8±10.0	NA	0.9
	S-insulin 60 min	16.0±12.4	19.1±11.5	NA	0.4
rs1890920	Individuals	687	16	1	
	Age (y)	45±13	46±14	NA	
	BMI (kg/m^2)	25.4±4.1	26.2±4.2	NA	
	Fasting P-glucose	5.47±0.66	5.56±0.67	NA	0.9
	Fasting S-insulin	6.18±3.64	7.13±3.87	NA	0.6
	S-insulin 30 min	19.1±11.6	18.2±10.1	NA	0.8
	S-insulin 60 min	15.8±12.3	18.0±11.2	NA	0.5
rs2419566	Individuals	463	225	21	
	Age (y)	44±13	46±12	43±13	
	BMI (kg/m^2)	25.5±4.0	25.0±3.8	26.5±3.6	
	Fasting P-glucose	5.47±0.65	5.47±0.68	5.54±0.61	0.9
	Fasting S-insulin	6.10±3.34	6.41±4.20	6.50±4.03	0.9
	S-insulin 30 min	19.2±11.2	19.2±12.2	20.2±15.7	0.9
	S-insulin 60 min	16.0±12.1	16.3±12.9	14.6±13.8	0.7

rs946940	Individuals	703	17	0	
	Age (y)	45±13	45±14	NA	
	BMI (kg/m^2)	25.4±3.9	26.0±4.1	NA	
	Fasting P-glucose	5.47±0.66	5.52±0.68	NA	0.8
	Fasting S-insulin	6.18±3.62	7.52±4.08	NA	0.15
	S-insulin 30 min	19.2±11.7	18.8±10.0	NA	0.8
	S-insulin 60 min	16.0±12.4	19.1±11.5	NA	0.2
rs1971596	Individuals	236	343	139	
	Age (y)	45±13	45±12	44±12	
	BMI (kg/m^2)	25.6±4.2	25.3±4.0	25.2±3.3	
	Fasting P-glucose	5.41±0.61	5.50±0.70	5.51±0.63	0.13
	Fasting S-insulin	6.28±3.26	6.09±3.68	6.38±4.12	0.7
	S-insulin 30 min	19.5±12.1	19.0±11.3	19.2±11.8	0.6
	S-insulin 60 min	16.2±12.8	15.9±12.7	16.1±10.8	0.9
rs17128356	Individuals	698	10	0	
	Age (y)	45±13	48±9	NA	
	BMI (kg/m^2)	25.4±3.9	25.3±3.1	NA	
	Fasting P-glucose	5.47±0.66	5.56±0.64	NA	0.8
	Fasting S-insulin	6.14±3.55	9.53±6.79	NA	0.04
	S-insulin 30 min	19.1±11.6	22.4±12.4	NA	0.4
	S-insulin 60 min	15.9±12.2	21.5±14.7	NA	0.2
rs521674	Individuals	397	258	45	
	Age (y)	45±13	45±13	44±12	
	BMI (kg/m^2)	25.5±4.0	25.6±3.9	24.2±2.4	
	Fasting P-glucose	5.50±0.67	5.44±0.64	5.51±0.59	0.5
	Fasting S-insulin	6.28±3.77	6.50±4.12	5.97±5.57	0.4
	S-insulin 30 min	20.5±13.6	19.6±13.9	14.6±7.9	0.01
	S-insulin 60 min	17.3±14.5	16.6±13.9	10.3±6.7	0.008
rs1800763	Individuals	653	7	0	
	Age (y)	45±13	48±10	NA	
	BMI (kg/m^2)	25.5±3.8	25.6±3.5	NA	
	Fasting P-glucose	5.48±0.66	5.53±0.76	NA	0.9
	Fasting S-insulin	6.19±3.58	9.98±7,41	NA	0.04
	S-insulin 30 min	19.3±11.7	21.8±13.2	NA	0.6
	S-insulin 60 min	16.2±12.4	21.6±15.9	NA	0.3
rs1800545	Individuals	622	77	1	
	Age (y)	45±13	46±10	NA	
	BMI (kg/m^2)	25.4±3.9	25.8±3.3	NA	
	Fasting P-glucose	5.48±0.67	5.46±0.60	NA	0.3
	Fasting S-insulin	6.12±3.38	6.87±5.19	NA	0.6
	S-insulin 30 min	19.1±11.7	19.8±11.4	NA	0.9
	S-insulin 60 min	15.8±12.0	17.4±13.9	NA	0.6
rs11195419	Individuals	604	100	1	
	Age (y)	45±13	47±12	NA	

	BMI (kg/m^2)	25.4±3.9	25.4±3.5	NA	
	Fasting P-glucose	5.47±0.65	5.54±0.70	NA	0.2
	Fasting S-insulin	6.14±3.41	6.56±4.79	NA	0.9
	S-insulin 30 min	19.1±11.8	19.6±10.9	NA	0.9
	S-insulin 60 min	15.8±12.0	17.4±13.6	NA	0.5
rs553668	Individuals	469	208	36	
	Age (y)	45±13	45±13	43±11	
	BMI (kg/m^2)	25.5±4.0	25.4±4.0	24.0±2.3	
	Fasting P-glucose	5.92±1.80	5.91±1.82	6.06±1.97	0.8
	Fasting S-insulin	6.31±3.96	6.08±3.01	5.18±2.48	0.3
	S-insulin 30 min	20.1±12.5	17.9±9.6	12.9±5.4	0.0009
	S-insulin 60 min	17.0±13.4	14.7±9.7	10.3±6.3	0.001
rs13306146	Individuals	683	26	0	
	Age (y)	45±13	39±12	NA	
	BMI (kg/m^2)	25.4±3.9	24.1±3.2	NA	
	Fasting P-glucose	5.47±0.66	5.37±0.74	NA	0.4
	Fasting S-insulin	6.20±3.65	5.68±2.82	NA	0.4
	S-insulin 30 min	19.2±11.7	17.5±9.1	NA	0.7
	S-insulin 60 min	16.0±12.3	13.6±8.2	NA	0.5
rs602618	Individuals	382	273	50	
	Age (y)	45±13	45±13	44±11	
	BMI (kg/m^2)	25.5±4.0	25.4±3.8	24.6±3.0	
	Fasting P-glucose	5.50±0.67	5.44±0.64	5.44±0.64	0.3
	Fasting S-insulin	6.25±3.64	6.25±3.75	5.25±2.63	0.3
	S-insulin 30 min	20.4±12.8	18.4±10.2	14.3±7.5	0.001
	S-insulin 60 min	17.1±13.4	15.6±11.0	10.2±6.5	0.002
rs4918621	Individuals	696	12	0	
	Age (y)	45±13	39±16	NA	
	BMI (kg/m^2)	25.4±3.9	25.0±3.5	NA	
	Fasting P-glucose	5.47±0.66	5.64±0.76	NA	0.4
	Fasting S-insulin	6.18±3.63	6.43±3.25	NA	0.9
	S-insulin 30 min	19.1±11.6	18.8±12.2	NA	0.8
	S-insulin 60 min	15.9±12.3	15.5±9.8	NA	0.9
rs4918625	Individuals	194	342	171	
	Age (y)	44±12	45±13	46±13	
	BMI (kg/m^2)	25.4±3.7	25.4±3.9	25.4±4.0	
	Fasting P-glucose	5.44±0.59	5.48±0.71	5,49±0.63	0.8
	Fasting S-insulin	5.72±2.96	6.44±3.86	6.17±3.77	0.2
	S-insulin 30 min	17.5±10.0	19.5±11.2	20.1±13.6	0.2
	S-insulin 60 min	13.8±10.2	16.5±11.6	17.2±15.1	0.06
rs1360866	Individuals	256	351	106	
	Age (y)	44±13	45±13	45±12	
	BMI (kg/m^2)	25.1±4.3	25.6±3.6	25.6±3.7	
	Fasting P-glucose	5.43±0.64	5.48±0.68	5,54±0.64	0.4
	Fasting S-insulin	6.09±3.55	6.16±3.60	6.64±4.02	0.5

	S-insulin 30 min	18.3±10.1	19.3±12.0	20.9±13.5	0.5
	S-insulin 60 min	14.9±10.7	16.6±12.8	16.8±14.4	0.4
rs7100191	Individuals	205	358	151	
	Age (y)	45±14	45±13	45±12	
	BMI (kg/m^2)	25.1±4.1	25.5±3.8	25.5±3.7	
	Fasting P-glucose	5.48±0.63	5.47±0.69	5,46±0.63	0.9
	Fasting S-insulin	6.24±3.45	6.29±4.00	5.92±2.93	0.9
	S-insulin 30 min	18.4±11.6	19.7±11.6	19.0±11.6	0.4
	S-insulin 60 min	15.7±12.3	15.9±12.2	16.7±13.0	0.6
rs2203616	Individuals	483	214	21	
	Age (y)	45±13	45±13	49±12	
	BMI (kg/m^2)	25.4±3.9	25.4±3.8	25.3±3.8	
	Fasting P-glucose	5.49±0.66	5.44±0.66	5,54±0.53	0.6
	Fasting S-insulin	6.17±3.68	6.26±3.62	6.72±2.99	0.5
	S-insulin 30 min	18.7±11.4	20.6±12.4	17.0±7.4	0.2
	S-insulin 60 min	16.2±12.6	15.9±12.2	14.5±7.4	0.9

		Genotypes			
Study	Phenotype	GG	GA	AA	P value
Botnia PPP (n=4681)					
SNP					
rs7911129	Individuals	4114	485	7	
	Age (y)	48±15	49±14	51±19	
	BMI (kg/m^2)	26.2±4.3	26.2±4.0	23.6±4.0	
	Fasting P-glucose	5.25±0.55	5.33±0.60	4.76±0.62	0.02
	Fasting S-insulin	6.60±5.57	6.66±4.76	5.07±2.00	0.3
	S-insulin 30 min	59.5±38.0	59.9±39.0	61.7±21.0	0.3
	S-insulin 120 min	32.9±34.1	33.0±37.0	29.2±18.6	0.3
rs1971596	Individuals	1435	2265	885	
	Age (y)	48±15	48±15	48±15	
	BMI (kg/m^2)	26.2±4.1	26.3±4.4	26.2±4.0	
	Fasting P-glucose	5.24±0.55	5.26±0.57	5.26±0.55	0.2
	Fasting S-insulin	6.51±5.66	6.68±5.75	6.60±4.46	0.2
	S-insulin 30 min	58.8±38.2	59.8±38.3	59.8±37.9	0.2
	S-insulin 120 min	32.8±32.7	32.4±32.6	34.6±41.4	0.3
rs553668	Individuals	3010	1354	174	
	Age (y)	48±15	48±15	48±15	
	BMI (kg/m^2)	26.4±4.3	26.0±4.1	26.0±4.2	
	Fasting P-glucose	5.26±0.56	5.26±0.55	5.22±0.57	0.7
	Fasting S-insulin	6.84±6.13	6.20±4.00	6.03±3.95	0.0004
	S-insulin 30 min	60.2±39.6	58.6±38.0	54.1±27.6	0.03
	S-insulin 120 min	33.6±34.7	31.6±34.5	30.7±27.8	0.03

rs602618	Individuals	2196	1856	382	
	Age (y)	48±15	48±15	49±15	
	BMI (kg/m^2)	26.4±4.3	26.1±4.2	26.3±4.3	
	Fasting P-glucose	5.26±0.56	5.25±0.56	5.27±0.56	0.4
	Fasting S-insulin	6.84±5.90	6.43±5.26	6.20±4.33	0.001
	S-insulin 30 min	60.3±39.2	59.0±37.5	56.9±31.8	0.09
	S-insulin 120 min	33.7±35.3	32.4±33.8	31.1±31.6	0.08
rs2203616	Individuals	2786	1602	211	
	Age (y)	48±15	49±15	47±15	
	BMI (kg/m^2)	26.2±4.2	26.3±4.3	26.1±4.0	
	Fasting P-glucose	5.24±0.55	5.28±0.56	5.24±0.61	0.1
	Fasting S-insulin	6.59±5.76	6.59±5.01	6.95±5.27	0.1
	S-insulin 30 min	59.6±37.7	59.0±38.4	62.8±40.8	0.3
	S-insulin 120 min	32.8±34.0	33.1±34.6	34.5±39.4	0.3

		Genotypes		
Study	Phenotype	GG	GA	AA
Case-Control				
SNP				
rs553668				
T2D cases (n=2830)	Individuals	1947	719	76
	Age (y)	63±11	63±11	62±11
	BMI (kg/m^2)	29.6±5.4	29.8±5.7	29.4±5.5
Controls (n=3740)	Individuals	2239	840	62
	Age (y)	57±6	57±6	57±6
	BMI (kg/m^2)	25.1±3.6	25.1±3.6	25.0±3.3

Table S3 Oligonucleotide sequences.
The microsatellite markers used to map N1I11 and N1I5, as well as gene-specific oligonucleotides. Oligonucleotides for *Adra2a* and *Pdcd4* were ordered as assays on-demand (Applied Biosystems).

Rat microsatellite markers		Forward primer sequence Reverse primer sequence
D1Swe8		5'-TCCTAGACAGTCAGGAGAAAC-3' 5'-ATTGCAGACTGTCCTGCTGG-3'
D1Rbm20-1		5'-CCTCAAGACAATACTAACTTACT-3' 5'-AACTGGATGCATCTAACTAATGA-3'
D1Got251		5'-GCAGCCAGAGAAGAGGGATTA-3' 5'-TGTAGCTCATGATTGAGACTGACC-3'
D1Gpam-1		5'-GTGGTGGTTGCTGAGGACTC-3' 5'-CTGCATTCCATAGCACCCTAT-3'
Gene description	Gene symbol	Forward primer sequence Reverse primer sequence Probe sequence
Hypoxanthine-guanine phosphoribosyltransferase	Hprt	5'-CAGCCCCAAAATGGTTAAGG-3' 5'-GCTCATTATAGTCAAGGGCATATCC-3' 5'-TCGAGAGGTCCTTTTCACCAGCAAGCTT-3'
ENSRNOG00000036577	36577	5'-GAGTTCCTTCTGTGGGCATT-3' 5'-TCGACTTCACTTCTGCCATC-3' 5'-CAGGAGCGAAAGCACTGTACCCA-3'
ENSRNOG00000032006 (Putative peptidoglycan-binding)	Lysmd3	5'-AGTGGATGTTAGCCACCATTCC-3' 5'-TGTTGGACCAATTGCATTTCC-3' 5'-AGTGGACTCTTCGCACTTACATC-3'
soc-2 (suppressor of clear) homolog (C. elegans)	Shoc2	5'-CGCTTAGCATCCGAGAGAAC-3' 5'-TGGGAGGTCTAGCAGCTCAT-3' 5'-CAGCTCCCTGCCGAGATCGG-3'

SUPPORTING REFERENCES AND NOTES

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