The rs553668 polymorphism of ADRA2A gene predicts the worsening of fasting glucose values in a cohort of subjects without diabetes. A population-based study

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The rs553668 polymorphism of the *ADRA2A* gene predicts the worsening of fasting glucose values in a cohort of subjects without diabetes. A population-based study

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Abstract

Aims   Single-nucleotide polymorphisms in the human *ADRA2A* gene have been associated with increased risk of Type 2 diabetes. The associations between the rs553668 polymorphism and fasting glucose concentrations both cross-sectionally and longitudinally after 6-year follow-up were evaluated in an adult Caucasian population-based cohort.

Methods   From a cohort of 1658 individuals, after excluding patients with diabetes, those who died and those whose blood samples were not available for genotyping, data of 1345 individuals were analysed.

Results   Subjects homozygous for the A allele showed significantly increased baseline fasting glucose values and a significant worsening of fasting glucose ($\beta = 0.48$; 95% CI 0.10–0.86) and insulin secretion ($\beta = -20.75$; -32.67 to -8.82 for homeostasis model assessment for β-cell function) at follow-up by using generalized estimating equations. Incidence of impaired fasting glucose and diabetes was almost twofold higher in subjects homozygous for the A allele (respectively: incident impaired fasting glucose 7.6–8.2, 16.1%, incident diabetes 1.7–2.3, 3.2% in GG, AG, AA carriers).

Conclusions   Our results suggested that the rs553668 polymorphism is associated with glucose worsening in subjects without diabetes at baseline.

Introduction

The stimulation of insulin release by glucose involves the closure of K^+ channels that are sensitive to the intracellular ATP concentration (K_{ATP}-channels), leading to membrane depolarization and the generation of Ca^{2+}-dependent action potentials [1]. The activation of α_{2a} adreneregic receptors, which are expressed in pancreatic β-cells, leads to an outward potassium current independent of the islet K_{ATP}-channels, which re-polarizes the β-cell sufficiently to suppress glucose-stimulated insulin secretion, thus mediating the adrenaline-mediated suppression of insulin secretion [1]. Over-expression of the *ADRA2A* gene (encoding the α_{2a} adreneregic receptors) was found to contribute to hyperglycaemia [2,3]. Single nucleotide polymorphisms in the human *ADRA2A* gene have been associated with reduced insulin secretion and increased risk of Type 2 diabetes [3,4]. Genome-wide association studies have investigated the single nucleotide polymorphism, rs10885122, of the *ADRA2A* gene, finding either decreased insulin response to oral glucose and worsening of fasting glucose [4,5] or no obvious effect on insulin secretion or sensitivity [6]. This single nucleotide polymorphism, however, is away from and uncorrelated with the rs553668 single nucleotide polymorphism, whose allele A has been recently associated with reduced insulin secretion and Type 2 diabetes [3].

To our knowledge, no longitudinal study on this polymorphism has been published.

We evaluated the associations between the rs553668 polymorphism and fasting glucose concentrations both cross-sectionally and longitudinally after 6-year follow-up, in a Caucasian population-based cohort of adults from Northern Italy.

Patients and methods

A population-based cohort of 1658 individuals was evaluated at baseline and after a mean follow-up period of 6.1 ± 0.34 years [7,8]. As hyperglycaemia in the diabetic range exerts deleterious effects on β-cell function, we excluded patients with diabetes at baseline. After excluding subjects with diabetes, those who died and those whose blood samples were not available for genotyping, data of 1345 individuals were collected.

Weight and height were measured after an overnight fast; waist circumference was measured by a plastic tape measure at the level of the umbilicus. Systolic and diastolic blood pressures were measured twice with a standard mercury sphygmomanometer in a sitting position, after at least 10 min of rest. Values reported are the mean of the two determinations. Individuals reporting a history of hypertension and current blood pressure medication use were defined as having hypertension, regardless of the blood pressure values measured.

In the morning, a venous blood sample was drawn to measure glucose, insulin, total cholesterol, HDL cholesterol and triglyceride levels. If the fasting serum glucose value was ≥ 6.1 mmol/l, a second fasting glucose determination was then performed. Serum glucose was measured by the glucose oxidase method using a Hitachi 911 Analyser (Sentinel Ch., Milan, Italy). Plasma triglycerides and HDL cholesterol were measured by enzymatic colorimetric assay (Hitachi 911 Analyser; Sentinel Ch.), the latter after precipitation of LDL and VLDL fractions using heparin–manganese chloride ($MnCl_2$) solution and centrifugation at 4 °C. Quantitative measurement of insulin in serum was performed with an immunoradiometric assay kit (Radim, Pomezia (RM), Italy). To better investigate differences in insulin levels at both the beginning of the study and the follow-up, insulin was measured in both samples simultaneously.

Clinical and laboratory methods have been described in detail previously [7,8]. Genotyping for *ADRA2A* rs553668 utilized the real-time allele discrimination method (Applied Biosystems, Foster City, CA, USA). On each reaction plate, at least two negative controls and known genomic DNA controls for optimal performance of allelic discrimination were included. The negative controls always showed no amplification, whereas the known genomic DNA controls always met the theoretical genotypes.

Patients gave their written informed consent to participate; all procedures were in accordance with the Declaration of Helsinki and approved by the local ethics committee.

With a total sample of 1345 subjects and a two-tailed 0.05 α-value, the study achieved > 90% power to detect a 0.12-mmol/l difference in fasting glucose values after follow-up between genotype groups.

All skewly distributed variables [insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment for β-cell function (HOMA-B) indexes] were logarithmically transformed. Cross-sectional associations among AG and AA genotypes (introduced in the model as dummy variables) and the baseline variables were evaluated by multiple regression models, after adjusting for baseline age, sex and BMI. Logistic regression was used to assess the association between each variant and incident impaired fasting glucose or diabetes, after adjustments for age, sex and BMI at follow-up minus baseline BMI (delta BMI).

Effects of genotypes to different clinical variables at the end of follow-up were estimated using a generalized estimating equations linear model, in order to consider the correlations within subjects. All analyses were performed by SAS software (SAS Institute, Cary, NC, USA).

Results

Characteristics of subjects evaluated for the rs553668 polymorphism did not differ from those of the whole cohort without diabetes. The observed genotype frequencies were in Hardy–Weinberg equilibrium ($P = 0.7$) (Table 1). Subjects who were homozygous for the A allele showed significantly increased baseline fasting glucose values. Glucose values at follow-up were, respectively, 5.4 ± 0.8, 5.5 ± 0.9 and 6.0 ± 1.5 mmol/l in the GG, AG and AA genotypes ($P < 0.001$ by ANOVA). Fasting glucose and HOMA-B values significantly changed in homozygous carriers of the A allele, by respectively increasing and reducing at follow-up in a generalized estimating equations linear model (Table 1). Incidence of impaired fasting glucose and diabetes was almost twofold higher in homozygous carriers of the A allele. The results did not change significantly after adjusting for the genotype for *TCF7L2* rs7903146, which is located 1.9 Mb downstream of *ADRA2A* [3] and which is the strongest candidate gene for Type 2 diabetes [6,8].

Blood pressure values, total cholesterol, HDL cholesterol and triglyceride levels were not significantly different among genotype groups (data not shown).

Discussion

Our results suggested that the rs553668 polymorphism in the *ADRA2A* gene is associated with fasting serum glucose values and supported the hypothesis that this single nucleotide polymorphism might be implicated in glucose worsening after 6-year follow-up. This might be attributable to reduced insulin secretion, as suggested by the significant reduction in HOMA-B values at follow-up in homozygous carriers of the A allele with respect to homozygous carriers of the G allele. Accordingly, reported impaired insulin granule docking at the plasma membrane and reduced β-cell exocytosis and impaired insulin secretion of animals and humans with over-expression of the α_{2a} adrenergic receptors, which, in humans, may be mediated by the rs553668 polymorphism, has been reported [2,3].

Our study might have been underpowered to detect differences in incidence of impaired fasting glucose or diabetes among the groups compared, as the prevalence of homozygosity for the defective A allele is low in this population-based cohort (2.3%). Nevertheless, in almost 20% of those individuals,

Characteristics of subjects at baseline and at follow-up by *ADRA2A* rs553668 genotypes

	GG $n = 924$ (68.7%)	AG $n = 390$ (29.0%)	AA $n = 31$ (2.3%)	AG Estimate† 95% CI	AA Estimate† 95% CI
Baseline*					
Males (%)	46.8	50.5	51.6		
CT + TT‡ (%)	60.4	58.7	71.0		
Age (years)	54.5 ± 5.7	54.1 ± 5.4	54.0 ± 5.6	−0.45 −1.17 to 0.22	−0.51 −2.53 to 2.02
BMI (kg/m^2)	26.4 ± 4.5	26.4 ± 4.3	27.8 ± 4.8	0.02 −0.50 to 0.54	1.38 −0.21 to 2.97
Waist (cm)	90.8 ± 12.6	91.4 ± 12.2	93.5 ± 14.3	0.60 −0.88 to 2.08	2.72 −1.77 to 7.21
Glucose (mmol/l)	5.5 ± 0.7	5.6 ± 0.7	5.9 ± 0.9§	0.08 0.002 to 0.16¶	0.32 0.09 to 0.56¶
Insulin (pmol/l)	40.8 (16.2)	40.2 (18.0)	42.0 (23.4)	0.018 −0.02 to 0.06	0.011 −0.10 to 0.12
HOMA-IR (mmol/l × μU/ml)	1.6 (0.7)	1.7 (0.9)	1.8 (1.3)	0.03 −0.01 to 0.07	0.07 −0.05 to 0.18
HOMA-B (μU ml^{-1}/mmoll^{-1})	77.0 (42.7)	77.4 (39.9)	74.7 (36.1)	−0.03 −0.08 to 0.02	−0.14 −0.30 to 0.02
Impaired fasting glucose (%)	19.4	19.2	22.6	1.00 0.91 to 1.10	1.08 0.44 to 2.66
At follow-up	Mean 95% CI	Mean 95% CI	Mean 95% CI	AG Estimate** 95% CI	AA Estimate** 95% CI
Delta BMI	0.22 0.10 to 0.34	0,24 0.09 to 0.39	0.16 −0.79 to 1.11	0.03 −0.47 to 0.54	1.35 −0.32 to 3.03
Delta waist	1.66 1.22 to 2.11	1.72 1.14 to 2.30	1.13 −1.48 to 3.74	0.48 −0.82 to 1.79	2.28 −2.16 to 6.72
Delta glucose	−0.07 −0.11 to 0.03	−0.11 −0.18 to 0.04	0.15 −0.31 to 0.62	0.06 −0.02 to 0.15	0.48 0.10 to 0.86¶
Delta insulin††	2.98 0.29 to 5.68	−0.23 −4.55 to 4.09	−2.19 −17.7 to 13.3	0.56 −3.04 to 3.95	1.41 −6.45 to 9.27
Delta HOMA-IR††	0.09 −0.03 to 0.21	−0.03 −0.24 to 0.17	−0.001 −0.82 to 0.81	0.07 −0.12 to 0.25	0.28 −0.19 to 0.76
Delta HOMA-B††	12.6 2.85 to 22.3	11.8 3.21 to 20.4	−0.46 −20.9 to 20.0	−3.86 −10.5 to 2.78	−20.75 −32.67 to −8.82¶
Incident impaired fasting glucose	7.6	8.2	16.1	1.09‡‡ 0.70 to 1.68	2.33‡‡ 0.86 to 6.28
Incident diabetes	1.7	2.3	3.2	1.34‡‡ 0.58 to 3.07	1.80‡‡ 0.23 to 14.2

*Data are expressed as mean ± SD for normally distributed variables, median (interquartile range) for skewly distributed variables and percentages for dichotomic variables.

†Estimate represents β (continuous variables) or odds ratio(impaired fasting glucose) for dummy variables, respectively, in linear and logistic regression models, after adjustment for age, sex and BMI, with GG being the reference category.

‡Allele CT + TT of *TCF7L2* rs7903146 polymorphism.

§$P < 0.05$ by analysis of variance (ANOVA) among the three genotype groups.

¶$P < 0.05$.

**Generalized estimated equation model, after adjustment for age, sex and delta BMI.

††Data available for 959 individuals.

‡‡Multiple logistic regression model, after adjustment for age, sex and delta BMI.

HOMA-B, homestasis model assessment of β-cell function; HOMA-IR, homestasis model assessment of insulin resistance.

hyperglycaemia (either impaired fasting glucose or diabetes) occurred at a rate twofold higher than in carriers of the G-allele. Therefore, these results reinforce previous cross-sectional data [3] in a longitudinal perspective also. If the longitudinal effects of the risk allele are linear, the long-term impact of this variant on glucose metabolism would be much more considerable.

Studies evaluating prospective associations of this polymorphism in larger cohorts are warranted in order to

better characterize pathways involved in the pathogenesis of Type 2 diabetes.

Competing interests

Nothing to declare.

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