

Influence of the ACE gene I/D polymorphism on insulin sensitivity and impaired glucose tolerance in healthy subjects

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ABSTRACT

Objective: Recent studies suggested that the blockade of the renin-angiotensin system (RAS) may be associated with metabolic benefits. However, data about the potential influence of the ACE I/D genotype on insulin resistance have been contradictory with studies of limited sample sizes. The purpose of this study was to investigate the relationship between the ACE gene I/D polymorphism and both insulin sensitivity and glucose intolerance in a large cohort of healthy subjects.

Research design and methods: 1286 participants in the RISC study had a 75-g oral glucose tolerance test and a hyperinsulinemic-euglycemic clamp to assess whole body insulin sensitivity.

Results: Age, BMI, waist, fat-free mass and physical activity did not differ by ACE genotype. Fasting glucose and insulin were similar among genotypes, but 2h glucose levels were higher in DD than in ID and II subjects (DD: 5.9 ± 1.7 ; ID: 5.7 ± 1.5 ; II: 5.6 ± 1.5 mmol/l, $p=0.004$). Participants with the DD genotype were more likely to have impaired glucose tolerance than those with the ID and II genotypes (13.1% vs 8.7%, $p=0.02$). Insulin sensitivity was lower in participants with the DD genotype than in those with the II genotype (136 ± 63 vs 147 ± 65 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kgffm}^{-1}\cdot\text{mM}^{-1}$, $p=0.02$). The presence of the D allele was associated with a trend, albeit not significant, for reduced insulin secretion during the OGTT ($p=0.07$).

Conclusions: The ACE I/D polymorphism is associated with whole body insulin sensitivity and with impaired glucose tolerance in our healthy population. These findings confirm potential interactions between the RAS and the glucose metabolism.

Recent studies have suggested that blockade of the renin-angiotensin system (RAS) is associated with a reduced incidence of type 2 diabetes (1). Most of these studies have been in hypertensive patients with a high prevalence of insulin resistance. These data have provided insights into possible interactions between the RAS and glucose metabolism. However, this topic remains controversial as the recent randomized DREAM trial did not show any significant reduction of the risk of 3-year incident type 2 diabetes with ramipril in participants with high plasma glucose levels or impaired glucose tolerance at inclusion (2). In this study, regression to normoglycemia was significantly more frequent in the ramipril group than in the placebo group and 2h glucose levels after a glucose load were significantly lower with ramipril, suggesting a possible subtle improvement in glucose metabolism with this ACE inhibitor (2).

The metabolic impact of increased RAS activity remains poorly understood. Effects on the insulin signalling cascade and hence peripheral insulin sensitivity have been suggested (3) but a specific effect on pancreatic β -cell function has also been proposed (4).

Angiotensin-converting enzyme (ACE) is a key enzyme in the RAS, modulating the synthesis of angiotensin II and inactivation of bradykinin. The ACE gene has an insertion/deletion (I/D) polymorphism, with the D allele being associated with higher ACE levels (5; 6). The relationship between the ACE I/D polymorphism and insulin sensitivity could therefore provide insight into the role of angiotensin II on glucose metabolism in humans. Previous data about the association of the ACE I/D genotype and insulin resistance have been contradictory but studies have included limited numbers of participants and/or only used surrogate markers of insulin sensitivity. The aim of our study was to investigate the relationship between the ACE I/D polymorphism and insulin sensitivity, as assessed by the

hyperinsulinemic-euglycemic clamp, in a large cohort of healthy men and women participating in the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease study) (7). In addition, as an oral glucose tolerance test (OGTT) was available, we also studied the association between the ACE I/D polymorphism and both fasting and 2h plasma glucose levels as well as beta cell function using the insulinogenic index and the interplay of insulin sensitivity and insulin resistance with the disposition index (8).

RESEARCH DESIGN AND METHODS

Study population. RISC is a prospective observational cohort study whose rationale, methodology and characteristics of the individuals recruited have been published (7; 9). Participants were recruited from the local population of 19 centres in 14 European countries, according to the following inclusion criteria: men and women, age between 30-60 years and clinically healthy. Initial exclusion criteria were: treatment for obesity, hypertension, lipid disorders or diabetes, pregnancy, cardiovascular or chronic lung disease, weight change of ≥ 5 kg in last 6 months, cancer (in last 5 years) and renal failure. Exclusion criteria after screening were: arterial blood pressure $\geq 140/90$ mmHg, fasting plasma glucose ≥ 7.0 mmol/l, 2-hour plasma glucose (on a 75-g OGTT) ≥ 11.0 mmol/l, total serum cholesterol ≥ 7.8 mmol/l, serum triglycerides ≥ 4.6 mmol/l, and ECG abnormalities. The present analysis is based on the 1,286 men and women who satisfied all criteria, whose clamp study passed the quality control check and for whom genomic DNA was available.

Lifestyle and medical history. Information was collected on personal and family medical history of CVD, stroke, hypertension and diabetes in first-degree relatives, as well as smoking and alcohol drinking habits and physical activity. Height, body weight, BMI, percent body fat and fat-free mass were evaluated by the TANITA bioimpedance balance (Tanita International Division, UK).

Obesity was defined as a BMI ≥ 30 kg/m². Waist girth was measured as the smallest circumference between iliac crest and rib cage. Sitting blood pressure and heart rate were measured three times after a 10-min rest (OMRON 705 cp, OMRON Healthcare Europe, The Netherlands).

Physical activity. Information on physical activity was collected with the 7-day International Physical Activity Questionnaire (IPAQ), a previously validated assessment tool for international studies that provides a comprehensive evaluation of daily physical activity habits (10; 11).

OGTT. Blood samples were taken before and 30, 60, 90 and 120 min into the OGTT, together with samples for central analysis of routine blood chemistry. Blood collected during the studies was separated into plasma and serum, aliquoted and stored at -20 °C for glucose and -80°C for lipids. Serum insulin was measured by a specific time-resolved fluoroimmunoassay (AutoDELFIA™ Insulin kit, Wallac Oy, Turku, Finland) (8). Plasma adiponectin was determined as previously described by an in-house time-resolved immunofluorometric assay (TR-IFMA) based on two antibodies and recombinant human adiponectin (R & D Systems, Abingdon, UK) (12). All standards and unknown samples were analysed in duplicate, with the exception of non-specific binding (NSB), which was analyzed in quadruplicate. The intra-assay coefficient of variation (CV) was <5 % and the inter-assay CV was <10 %.

Insulin clamp. On a separate day within one month of the OGTT, a hyperinsulinemic-euglycaemic clamp was performed. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 pmol.min⁻¹.m⁻² simultaneously with a variable 20% dextrose infusion adjusted every 5-10 min to maintain plasma glucose level within 0.8 mmol/l ($\pm 15\%$) of the target glucose level (4.5-5.5 mmol/l). The clamp procedure was standardised across centres; the data from each clamp study were immediately transferred to the coordinating centre where

they were underwent quality control scrutiny according to pre-set criteria.

Local Ethics Committee approval was obtained by each recruiting centre. Volunteers were given detailed written information and signed a consent form.

Data analysis. Glucose tolerance was categorised into normal, impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) according to the last ADA criteria. The Homeostasis Model Assessment (HOMA) index of insulin resistance was calculated. Insulin sensitivity was expressed as the ratio of the M value— averaged over the final 40 min of the 2-hour clamp and normalised by the fat-free mass – to the mean plasma insulin concentration measured during the same interval (M/I, in units of $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kgffm}^{-1}\cdot\text{mM}^{-1}$).

The insulinogenic index of beta cell function was calculated as the ratio of the increment in insulin concentration over the first 30 minutes of the OGTT to the increment in glucose concentrations over the same time period. A disposition index (insulin secretion/insulin resistance index) was calculated as the product of the insulinogenic index and the insulin sensitivity index obtained from the clamp (expressed after division by 1000 for simplification) (8).

ACE I/D polymorphism. Genomic DNA was extracted using a Nucleon BACC2 kit (Tepnel Life Sciences Plc, Manchester, UK). The ACE gene I/D polymorphism was genotyped by PCR using the forward primer 5' CTG GAGACCACTCCCATCCTTTCT-3' and reverse primer 5' GATGTGGCCATCACATTCGTC AGAT 3' (13). A 20 μl PCR reaction consisting of a final concentration of: 0.8 μM of each primer, 200 μM of each dNTP, 0.15 U of AmpliTaq Gold (Applied Biosystems, Warrington, UK), 1 \times PCR buffer, 1.5 mM MgCl₂ and 50 ng of genomic DNA was performed. Thermal cycling conditions were: 5 min at 95°C then 30 cycles consisting of 1 min at 94°C, 1 min at 67°C and 2 min at 72°C, with a final extension step at 72°C for 5 min. PCR products were separated on a 2% agarose gel

and expected fragment sizes were 190 bp for the D allele and 490 bp for the I allele. Because the D allele in heterozygous samples is preferentially amplified, each sample found to have the DD genotype was subjected to a second PCR amplification using primers that recognise the insertion specific sequence (13). The PCR reaction produces a 335 bp fragment in the presence of the I allele and no product in DD homozygotes. Of the genotyped samples, 5% were duplicates and there was at least 1 negative control per 96 well DNA plate. The accuracy of the genotyping was determined by the genotype concordance between duplicate samples. We obtained a 100% concordance between the genotyped duplicate samples.

Statistical analysis. The data are expressed as mean \pm SD unless otherwise specified, categorical data as percentages. Variables which were not normally distributed were log transformed before the analyses. Variables were compared between genotypes using t-tests. The relationship between metabolic variables and the ACE genotype was assessed by linear regression analysis with an additive genetic model, firstly without and then with adjustment for age, sex, centre, physical activity and waist circumference; dominant and recessive genetic models were also studied with adjustment for the same covariates. Logistic regression analysis was used to assess the relationship between impaired glucose tolerance and ACE genotypes after correction for the same covariates. Statistical analyses used StatView for Windows (version 5.0, SAS Institute Inc.,NC).

RESULTS

A total of 1286 subjects (579 men and 707 women) with a mean age of 43.8 ± 8.3 years were studied. The main clinical characteristics of the population studied are presented in Table 1. The mean BMI was 25.5 ± 4.1 kg/m² with 13.2% being obese. The ACE gene I/D genotype distribution is presented in Table 1. The frequencies of the I and D alleles were 0.45 and 0.55,

respectively and the genotype frequency distributions were in Hardy-Weinberg equilibrium.

The ACE genotype was not associated with age, sex, BMI, waist or hip circumference nor with fat free mass. The level of physical activity and the prevalence of family history of type 2 diabetes did not differ by ACE genotype. Similarly, fasting glucose was similar among genotypes (Table 2).

Before adjustment for covariates, post load glycemia at 2h differed significantly according to ACE genotypes with higher mean values in DD compared to II ($p=0.006$) or ID ($p=0.07$), but 2h glycemia did not differ between II versus ID participants ($p=0.14$). This association between the ACE genotype and 2h glycemia persisted after correction for age, sex, centre, physical activity and waist circumference (Table 2). There were no interactions between the effects of sex or elevated BMI and the ACE genotype.

In both univariate and multivariate analyses, the ACE genotype was not significantly related to fasting glycemia, insulin and proinsulin levels nor to plasma adiponectin concentrations (Table 2). The presence of the D allele was associated with a trend, albeit not significant ($p=0.07$), for reduced insulin secretion during the first 30 minutes of the OGTT, as assessed by the insulinogenic index (Table 2).

Insulin sensitivity, as assessed by the M/I value from the clamp, was lower in subjects with the DD genotype than in those with the II genotype ($p=0.02$) and in those with the II genotype as compared to the ID genotype ($p=0.05$) with no differences between ID and DD genotypes ($p=0.47$). The association between the ACE I/D genotype and insulin sensitivity persisted after adjustment for age, sex, centre, physical activity and waist circumference (Table 2). For the disposition index, there was also a significant difference between genotypes, before and after adjustment on these factors.

We assessed the genotype effect of ACE I/D polymorphism on metabolic traits

according to different genetic models of inheritance (additive, dominant and recessive). As presented in Table 2, the influence of the ACE genotype on 2h glycemia was consistently observed in all genetic models tested. For both insulin sensitivity (M/I value) and the disposition index the relationship was significant in either the additive or dominant, but not in the recessive model.

There was a graded increase in the prevalence of impaired glucose tolerance (IGT) according to the number of D alleles (Table 2). In a logistic regression taking into account age, sex, centre, physical activity and waist circumference, the DD genotype conferred an increased risk of having IGT as compared to the other genotypes [OR: 1.52; (95% CI: 1.01-2.28), $p=0.04$]. The highest odds ratio for IGT was observed when comparing the DD versus the II genotype [OR: 2.02; (95% CI: 1.09-3.74), $p=0.02$]. The ID genotype did not have a significantly higher odds for IGT as compared to the II genotype [OR: 1.47; (95% CI: 0.81-2.65), $p=0.20$].

The significant relationship between the ACE I/D genotype and the 2h glycemia persisted after inclusion of either the M/I value or the insulinogenic index as covariates in the multivariate model. However, if we included both the M/I value and the insulinogenic index (or the disposition index), the relationship between the ACE genotype and 2h glycemia was no longer significant. In each of these analyses, the M/I value appeared to be a stronger determinant of 2h glycemia (or IGT) than the insulinogenic index.

CONCLUSIONS

The results of the present study show that the ACE I/D polymorphism is associated with whole body insulin sensitivity and with 2h glucose levels in our healthy population. The presence of the D allele confers an increased risk of having glucose intolerance in this normotensive, non-diabetic population. Specifically, those who are homozygous for the D allele have higher 2h

glycemia, lower glucose utilization and a lower disposition index than those who are heterozygous or homozygous for the I allele.

To our knowledge, the present study is the first to report an association between the ACE I/D genotype and impaired glucose tolerance in a non-diabetic healthy population.

The existence of an association between the ACE I/D polymorphism and glucose metabolism has been controversial so far. Most of the studies reported no relationship between the I/D variant of the ACE gene and surrogate markers of insulin-resistance, such as fasting blood glucose and insulin levels or the HOMA index (14-16). This is consistent with our data that showed no significant differences in fasting blood glucose or insulin levels according to ACE genotypes. In the present study, only the use of the OGTT and the euglycemic clamp led to the detection of differences in glucose metabolism across genotypes. One previous study showed in 66 overweight women, that those with the II genotype were more insulin-resistant than those with the DD genotype (17). However, this study was restricted to women and only 9 subjects presented with the II genotype. The discrepancies between our findings and this previous report may therefore relate to differences in the type of population studied and in the sample size.

Previous studies showed higher glycemia during an OGTT in diabetic or non-diabetic subjects with the DD genotype, in agreement with the present findings (14; 18) but these studies did not detect differences in the prevalence of glucose intolerance according to genotypes owing probably to smaller sample sizes.

The D allele of the ACE gene has been associated with higher ACE levels (5; 6; 19). Our data that show higher 2h glucose levels in those with the DD genotype are therefore consistent with the results of the recent randomized DREAM trial in which 2h glucose levels were significantly lower with ramipril treatment as compared to the placebo group after a median follow-up of 3 years (2). Altogether, these findings are

consistent and support a role for ACE activity in the regulation of post-load glucose homeostasis.

The mechanisms by which insulin sensitivity may differ according to ACE genotypes remain poorly characterised. Haemodynamic effects of angiotensin II may contribute to reduce the perfusion of skeletal muscle and therefore glucose utilization (20). In addition, at the cellular level, angiotensin II induces alterations in insulin signalling cascade by stimulating multiple serine phosphorylation events and inducing oxidative stress (3). Animal studies suggested that skeletal muscle fibre composition is related to insulin resistance, and that modulation of ACE activity may modulate the ratio of type 1 muscle fibres (21). Heterozygous mutant (ACE+/-) mice with reduced ACE activity had significantly more capillaries around skeletal muscle fibers than normal wild type mice, supporting an effect of ACE activity on muscle capillary density, which may directly impact on local glucose uptake (22). Whether the ACE I/D polymorphism has an effect on muscle capillary density in humans is still not known. The I allele has been associated with an increased percentage of slow-twitch type I fibers in human skeletal muscle, suggesting a potential impact of ACE genotype on the structure and metabolism of muscles in humans (23). However, the relevance of these findings to explain differences in insulin sensitivity remains to be established. We cannot exclude an effect of the ACE I/D genotype on endogenous hepatic glucose production that could also contribute to explain the differences of 2h glucose levels.

In the present report, the presence of the D allele was also associated with a trend, albeit not significant, for reduced insulin secretion during the first 30 minutes of the OGTT, using either an additive or the dominant models of inheritance. This is consistent with previous data (18) but the present study is the first one to show an association between the ACE I/D genotype and the disposition index, suggesting an impact of this genotype on β -cell function.

The presence of the RAS in human β -cells has been described (4; 24). Acute infusion of angiotensin II impairs first phase insulin secretion, possibly through alterations in intraislet blood flow (4). These findings on the effects of the ACE genotype on insulin secretion may represent therefore an additional mechanism involved in the association between ACE I/D polymorphism and both 2h glucose levels and the risk of IGT. This association between the disposition index and the ACE I/D genotype is of interest given the recent observation that the interplay between insulin resistance and insulin secretion is a strong predictor of future development of type 2 diabetes (8).

The strengths of the present study of the RISC cohort are the large population of normotensive and healthy subjects, the use of the gold-standard methodology for measurement of insulin sensitivity with centralised laboratory assays and continuous quality control of data (7). In addition, we considered both insulin resistance and insulin secretion indices. However, the cross-sectional nature of our study does not allow us to draw conclusions on the causality of the link between ACE I/D genotype and glucose metabolism.

In our large population of healthy subjects, the deletion polymorphism of the ACE gene, a marker of higher ACE activity, was significantly associated with impaired glucose tolerance, reduced glucose utilization in a hyperinsulinaemic euglycaemic clamp and a lower disposition index. These findings therefore suggest a role for local RAS in glucose metabolism and open some new perspectives for both prediction and prevention of type 2 diabetes. Intervention studies are needed to determine whether metabolic effects of the blockers of the RAS may differ according to the ACE I/D genotype.

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Further information on the RISC Study and participating centres can be found on www.egir.org.

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TABLE 1. Clinical characteristics of the EGIR-RISC study participants according to ACE genotype

	Overall	II	ID	DD
No. of participants	1286	243	662	381
Age (years)	44 ± 8	43 ± 8	44 ± 8	44±8
Sex (% of men)	45	47	44	44
BMI (kg/m ²)	25.5 ± 4.1	25.6 ± 4.1	25.4 ± 4.0	25.7 ± 4.1
Waist circumference (cm)	86.6 ± 13	87.4 ± 12	86.1 ± 12	87.1 ± 14
Hip circumference (cm)	100.3 ± 9	101.0 ± 9	100.1 ± 9	100.3 ± 9
Family history of diabetes (%)	27.5	24.1	28.8	27.3
Fat mass (kg)	21.0 ± 8.9	21.1 ± 9.0	20.8 ± 8.7	21.2 ± 9.2
Fat free mass (kg)	53.8 ± 11.4	54.1 ± 10.9	53.6 ± 11.6	54.2 ± 11.6

TABLE 2. Indices of insulin sensitivity and glucose tolerance according to ACE genotype

ACE genotype	<i>P value</i>						
	II	ID	DD	Additive unadjusted	Additive adjusted	D dominant adjusted	D recessive adjusted
Fasting glycemia (mmol/l)	5.1 ± 1.1	5.1 ± 0.6	5.1 ± 0.6	0.80	0.47	0.56	0.56
Glucose at 120 min (mmol/l)	5.6 ± 1.5	5.7 ± 1.5	5.9 ± 1.7	0.006	0.001	0.004	0.015
Fasting insulin* (pmol/l)	31 (19-42)	31 (19-43)	31 (20-42)	0.67	0.58	0.43	0.87
Fasting proinsulin* (pmol/l)	6 (4-8)	6 (4-8)	6 (4-8)	0.72	0.54	0.24	0.94
Insulinogenic index* (pmol/mmol)	78 (43-112)	77 (43-111)	71 (40-102)	0.12	0.07	0.32	0.07
Impaired fasting glucose (%)	17.0	17.0	19.9	0.30	0.53	0.68	0.56
Impaired glucose tolerance (%)	7.5	9.2	13.1	0.018	0.019	0.07	0.04
Adiponectin* (mg/l)	7.3 (5.0-9.5)	7.9 (5.6-10.2)	7.6 (5.1-10.1)	0.64	0.43	0.27	0.80
HOMA-IR*	0.95 (0.5-1.3)	0.97 (0.6-1.4)	0.95 (0.6-1.3)	0.68	0.54	0.42	0.81
Disposition index*	10.5 (5.3-15.7)	9.3 (4.6-14.0)	8.9 (4.5-13.3)	0.02	0.01	0.02	0.07
M/I value*	145 (99-190)	128 (85-171)	124 (85-163)	0.06	0.04	0.019	0.29

M/I is expressed as ($\mu\text{mol}/\text{min}/\text{kgFFM}$)/(nmol/l). Adjustment is done for sex, age, centre, physical activity and waist circumference according to alternative genetic models of inheritance (additive, D dominant and D recessive).

*Expressed as the median (interquartile range).