Is the influence of variation in the ACE gene on the prospective risk of Type 2 diabetes in middle-aged men modified by obesity?

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Abstract

There is strong evidence for the presence of a functional renin–angiotensin system in diabetogenic tissues, and ACE (angiotensin-converting enzyme) inhibitors may improve glucose metabolism in those individuals at high risk of developing T2DM (Type 2 diabetes). In the present study, we tested the hypothesis that subjects with genetically lower plasma and tissue ACE activity, because of their ACE [I/D (insertion/deletion)] genotype, would have a lower risk of T2DM in 2642 healthy middle-aged Caucasian men (mean age, 56 years) followed-up for 15 years. Obesity was the strongest predictor of T2DM, with an HR (95% CI) [hazard ratio (95% confidence interval)] of 3.74 (2.66–5.26) (P < 0.0001). Overall there was no association between ACE genotype (II homozygotes, n = 623; and D allele carriers, n = 2019) and risk of T2DM, and although in lean men there was no genotype difference in risk in D allele carriers compared with II homozygotes [adjusted HR = 0.75 (95% CI, 0.46–1.22)], in obese (body mass index > 30 kg/m²) men the risk of T2DM was higher [adjusted HR = 4.26 (95% CI, 1.30–13.93)] with a genotype–obesity interaction of P = 0.01. A similar pattern of risk was seen by re-analysis of a previously published case-control study, where D allele carriers had a non-significant 1.30 (0.97–1.74)-fold higher risk of developing T2DM than II homozygotes when non-obese, but a 1.79 (1.17–2.72) (P = 0.007)-fold higher risk when obese. Further prospective studies are needed to confirm these findings. The ACE D allele may worsen glucose metabolism, which could raise the prospective T2DM risk in obese men, but not in lean men. In obesity, adipose tissue undergoes inflammatory infiltration and the subsequent higher levels of pro-inflammatory angiotensin II may explain this association.

Introduction

It is likely that ACE [Ang (angiotensin)-converting enzyme] plays a role in the pathogenesis of T2DM (Type 2 diabetes), as a renin–Ang system exists in the relevant metabolically active tissues, namely adipose tissue, skeletal muscle and pancreas [1]. AngII, the product of the action of ACE on AngI, is known to be pro-inflammatory and cause insulin resistance [2]. Intervention to disrupt the renin–Ang system, by the administration of ACE-inhibitors or ARBs (AngII receptor blockers), has been shown to prevent the development of T2DM in high-risk

Key words: angiotenin II, angiotensin-converting enzyme gene (ACE gene), body mass index (BMI), gene–environment interaction, inflammatory state, obesity, Type 2 diabetes.

Abbreviations: Ang, angiotensin; ACE, Ang-converting enzyme; ARB, AngII receptor blocker; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; D, deletion; HR, hazard ratio; I, insertion; NPHSII, Second Northwick Park Heart Study; OR, odds ratio; SBP, systolic BP; SNP, single nucleotide polymorphism; T2DM, Type 2 diabetes; UDACS, University College London Diabetes and Cardiovascular Disease Study.

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individuals in a meta-analysis [3]. This raises the possibility that these individuals with genetically lower levels of ACE may be protected from the development of T2DM.

Serum and tissue ACE activity is strongly associated with a common variant in the ACE gene, with the presence of an insertion (I) of a 287 bp fragment in intron 16, associated with lower ACE activity, and the deletion (D), associated with higher ACE activity [4,5]. The impact of the ACE I/D polymorphism on T2DM risk has been studied widely with varying results. There are, however, a number of studies in which no association has been found between this genotype and T2DM [6,7]. The D allele was associated with a higher T2DM risk [OR (odds ratio) = 1.52, P = 0.02] in a case-control study [8]. In those with T2DM, D allele carriers have poorer outcomes, with a higher risk of myocardial infarction and renal disease [9], and a worse response when starting on an intensive hypoglycaemic medication regime [10]. Paradoxically, the D allele has been associated with a higher insulin sensitivity [11]. The majority of these studies are limited by being cross-sectional and we therefore set out to examine the hypothesis that men with the low ACE II genotype would be protected from developing T2DM in a prospective 15 year follow-up study.

Recently, studies have suggested that the ACE gene modulates the development of disease phenotypes through interaction with key environmental factors. Interaction between smoking and the ACE polymorphism has been associated with a higher cardiovascular disease mortality at a younger age [12]. Interaction between the ACE D allele and hypertension, leading to a higher risk of heart failure, has also been reported [13]. We are not aware of any reported ACE gene–environment interactions influencing T2DM; however, combined SNP (single nucleotide polymorphism) interactions or haplotype effects have been reported in diabetic nephropathy [14,15]. Only a few gene–environment interactions have been reported so far in T2DM, with an interaction between PPARγ Pro12Ala [where PPARγ encodes PPARγ (peroxisome-proliferator-activated receptor γ)] and obesity on T2DM risk [16], whereas an interaction between lifestyle intervention and a TCF7L2 SNP (where TCF7L2 encodes transcription factor 7-like 2) on T2DM risk has been reported [17]. For T2DM, the major environmental determinant of risk is obesity [18]. In view of the raised inflammatory state in obesity and AngII being pro-inflammatory, we looked for interactions between obesity and ACE genotype in determining prospective T2DM risk.

**MATERIALS AND METHODS**

Subjects were recruited from the prospective NPHSII (Second Northwick Park Heart Study), as detailed elsewhere [19]. The study was approved by the Institutional Ethics Committees and performed in accordance with the Declaration of Helsinki. Briefly, 3012 unrelated healthy Caucasian middle-aged male subjects were enlisted from nine U.K. general medical practices throughout the U.K. and prospectively followed-up for development of CHD (coronary heart disease) from 1989. Self-report by questionnaire identified those with T2DM at baseline (n = 76), who were excluded from the prospective analysis. Subjects requiring insulin, oral hypoglycaemics or any other cardiovascular medication were excluded from entry into NPHSII [19]. New cases were identified by medical practice note search for physician-diagnosed and -treated T2DM according to current national guidelines. The reporting of T2DM was done thoroughly and systematically.

Out of the initial 3012 recruits, 76 with T2DM were excluded and, in 230, DNA could not be extracted. DNA was therefore available for 2706 eligible men, and genotypes were obtained in 2642 subjects (97.5 %). There were only significant differences in SBP (systolic BP (blood pressure)) and fibrinogen between the group of 230 and 2706 eligible samples, but the absolute differences were small and unlikely to have had an impact (see Supplementary Table 1 at http://www.clinsci.org/cs/113/cs1130467add.htm). There were no differences in baseline characteristics between the 64 drop-outs and the 2642 subjects with ACE genotype (results not shown). Two of those individuals without T2DM and one with T2DM did not have height recorded and, thus, there are missing data on BMI (body mass index) in these three individuals. Genotypes were determined by leucocyte DNA PCR using primers and conditions for ACE described previously [20], and were resolved using MADGE (microarray diagonal gel electrophoresis) for approx. 87 % of the sample, and using Taqman, primers and probes for rs4341 [in complete LD (linkage disequilibrium) with ACE I/D polymorphism] as described previously [21] for the remaining samples. The genotype was determined by two independent technicians blinded to the subject outcome. One DNA array (94 samples) was genotyped for both ACE I/D and rs4341 polymorphisms with 100 % concordance. Obesity was defined as BMI > 30 kg/m². Results are presented as HRs (hazard ratios) obtained with their corresponding 95 % CIs (confidence intervals). A dominant genetic model was assumed based on the results from our previous cross-sectional study and other studies [8,10,13].

**Statistical analysis**

Statistical analysis was performed using Intercooled STATA (version 8.2). Baseline characteristics were transformed to a normal distribution as appropriate. HRs were obtained from Cox proportional hazard models with their corresponding 95 % CIs. All models included age as a covariate, and were stratified by general medical practice in order to take into account modest
differences in the baseline hazard by recruitment site. Interactions were tested as deviations from multiplicative effects in the Cox model using the likelihood ratio test. Scaled Schoenfeld residuals were used to verify that relative hazards were constant over time. Frequencies were compared by \( \chi^2 \) test.

**RESULTS**

ACE genotype data were obtained from 2642 men. After 15 years of follow-up, 153 men with ACE genotype developed T2DM. The remaining 2489 men were alive and had not developed T2DM. Baseline BMI, obesity, CRP (C-reactive protein), triacylglycerol (triglyceride), cholesterol and BP were all associated with an increased risk of developing T2DM (Table 1). Alcohol consumption was not different between those who developed T2DM and those who did not. The highest risk was that associated with obesity [HR, 3.74 (95% CI, 2.66–5.26); \( P < 0.0001 \)]. When a stepwise model was used to determine which of these variables were independently associated, BMI remained the most significant predictor, with an age- and general-medical-practice-adjusted HR of 1.91 (95% CI, 1.64–2.24; \( P < 0.0001 \)). There was no significant difference between those who developed T2DM and those that did not with regard to ACE genotype distribution (\( P = 0.48 \)) or D allele frequency (\( P = 0.87 \)) (Table 1). Distribution of genotypes was as expected for Hardy–Weinberg equilibrium. HR (adjusted for age and general medical practice) for developing T2DM for DD + ID compared with II was 0.91 (95% CI, 0.63–1.32; \( P = 0.63 \)). To eliminate the possibility of confounding, we confirmed that there were no significant differences in baseline BMI, BP, lipid parameters, age, CRP or alcohol consumption between the different genotype groups.

Since obesity was the strongest risk factor for development of T2DM and in view of the potential for escalating the inflammatory burden in obese individuals, the interaction between ACE genotype and obesity on 15-year risk of developing T2DM (adjusted for age and general medical practice) was examined. In non-obese men, D allele carriers had a non-significantly lower risk of T2DM [HR, 0.69 (95% CI, 0.45–1.06) for ID compared with II], whereas in obese men the association was reversed [HR, 2.11 (95% CI, 0.95–4.69) for ID compared with II] with a significant genotype–obesity interaction (\( P = 0.02 \)). Figure 1 shows that, when adjusting for triacylglycerol, CRP and SBP [the three strongest predictors of T2DM (Table 1) which may also be causal], the risk of T2DM in obese men was similarly higher in D allele carriers compared with II homozygotes [HR, 4.26 (95% CI, 1.30–13.93)], whereas in lean men there was no genotype difference in risk [HR, 0.75 (95% CI, 0.46–1.22)], with an overall interaction of \( P = 0.01 \). A Kaplan–Meier plot (Figure 2) demonstrates that the development of T2DM in obese subjects occurred much earlier than in non-obese men, with the curve for obese D carriers separating from obese II homozygotes between 7–10 years of follow-up. Figure 2 also shows that, after 15 years, those subjects with the lowest T2DM risk were the non-obese D allele carriers. When analysis was carried out using BMI as a continuous variable, HR for a one-S.D. increase in BMI was found to be higher, but not significantly so, with ID/DD compared with II [HR, 2.18 (95% CI, 1.83–2.61) compared with 1.73 (95% CI, 1.27–2.36); \( P = 0.20 \)]. However, there was a clear divergence of risk when BMI > 30kg/m² (Supplementary Figure 1 at http://www.clinsci.org/cs/113/cs1130467add.htm).
Figure 2 Kaplan–Meier plot for T2DM by ACE genotype and obesity

Figure 3 Categorical model for the interaction of BMI with ACE genotype on the prospective risk of T2DM

The reference group is specific for each BMI category and is the ratio of the number of subjects with the II genotype who develop T2DM after 15 years to the total number of subjects with the II genotype within the same BMI category. Categories of BMI were as used in a previous analysis [22]. HRs were adjusted for age, general medical practice, triacylglycerols, CRP and SBP. Interaction P = 0.01. Error bars are 95 % CIs. [Number of subjects/number developing T2DM].

Analysis using BMI in four categories using cut-off values reported previously [22] showed a non-linear pattern of interaction with a threshold effect evident, with the D allele being associated with a risk of T2DM (adjusted for age, general medical practice, triacylglycerols, CRP and SBP) only at high BMI (genotype–BMI interaction, P = 0.02), as shown in Figure 3.

On the basis of these findings, the results in our previous cross-sectional study [8] were re-examined, where those subjects with T2DM in UDACS (University College London Diabetes and Cardiovascular Disease Study) [23] were compared with those without T2DM in NPHSII (controls). Analysis was confined to Caucasian subjects with T2DM (n = 605, of whom 574 were successfully genotyped for the ACE I/D gene variant). T2DM was defined as those subjects who fulfilled WHO (World Health Organization) criteria and not requiring insulin within 12 months of diagnosis. In non-obese patients, there was no significant difference in risk of T2DM between D allele carriers and II homozygotes, but in obese men the risk of T2DM was significantly higher in D allele carriers [OR, 1.79 (95 % CI, 1.17–2.72); P = 0.007], although the genotype–obesity interaction was not significant (P = 0.22) (Figure 4). A similar pattern of association was present when analysis was confined to only male cases in UDACS, with no genotype difference in risk in non-obese men [OR, 1.28 (95 % CI, 0.89–1.85) for D allele carrier compared with II], but in obese men, OR of T2DM for D allele carriers compared with II subjects was 2.26 (95 % CI, 1.31–3.91) with a genotype–obesity interaction of P = 0.09.

DISCUSSION

On the basis of the protective effect of ACE inhibitors and ARBs on the development of T2DM in a meta-analysis of large clinical trials [3], the present study tested the hypothesis that ACE II subjects, with genetically determined lower ACE levels, would have a lower rate of development of T2DM. This effect was confined to obese men where D allele carriers had a significantly greater risk than their II counterparts, but in non-obese men there was no difference in genotype risk. Possession of the D allele, therefore, may be increasingly harmful when individuals are obese. Further examination suggested that this was not a linear effect of BMI on risk, but rather the effect only became significant once the individual had a BMI > 30 kg/m². In a cross-sectional study of T2DM published previously [8], re-analysis of the data demonstrated a trend where D allele carriage was associated with a risk of T2DM only in obese subjects.

The mechanism of the ACE gene variant and its relationship with obesity in the pathogenesis of T2DM is currently unclear, but is likely to be due to the impact...
of the different levels of plasma and tissue ACE that will be present constitutively, with the II subjects having approx. 40% lower levels than DD subjects [4,5]. The higher serum and tissue ACE associated with the D allele would lead to higher AngII levels. Renin–Ang systems are present in the circulation and in several tissues in which glucose metabolism are controlled, including skeletal muscle, pancreas and adipose tissue. There is now considerable evidence for AngII promoting the development of T2DM through a number of mechanisms, including interfering with insulin signalling, pro-inflammatory effects on tissue beds causing endothelial dysfunction, inhibiting adipocyte differentiation and causing β-cell dysfunction via oxidative stress [24–26]. It is likely that the higher AngII levels in D allele carriers would have a bigger impact in the obese state, where there is already a greater inflammatory and diabetogenic burden. It is, however, important to note the wide 95% CIs for D allele carriers (adjusted for age, general medical practice, triacylglycerols, CRP and SBP). This would be in keeping with a small effect of the ACE (I/D) polymorphism on the risk of developing T2DM, which ties in with conclusions from the DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) trial [27], where ramipril did not reduce incidence of T2DM in high-risk individuals but suggested a small benefit in glucose metabolism.

Adjustments of the ACE risk effects were made for SBP, triacylglycerols and CRP because, with BMI, these were the strongest predictors of T2DM development in this cohort. Such adjustments did not materially alter the genotype risk pattern seen. All three factors may be involved in the same causal pathway in T2DM pathogenesis. SBP can directly activate the renin–Ang system [28]. Non-esterified fatty acids (from triacylglycerol hydrolysis) directly inhibit insulin signalling [29], and excess intracellular triacylglycerol promote increased oxidative stress and inflammation which can cause insulin resistance [30]. The properties of AngII affecting insulin signalling and causing oxidative stress would directly affect this. CRP may act together with AngII in promoting insulin resistance, again through direct and indirect effects on insulin signalling and oxidative stress [31].

There are several limitations of the present study. The method of identification of NPHSII men with T2DM, by medical record search, is unlikely to include any false-positive diagnoses, but, in the absence of a full recall for fasting glucose testing, some T2DM subjects may be misclassified as healthy. This would result in an underestimate of the 15-year incidence of T2DM, reducing the ability to detect an effect of the polymorphism, and would not confound the genetic association seen.

The lack of plasma glucose and serum insulin data prevents an exploration of whether these parameters are affected by the ACE I/D genotype, in light of the potential detrimental impact of AngII on insulin signalling and glucose metabolism. Background diet and physical activity data would have also been useful, as they could affect the development of T2DM. Measurement of plasma AngII (or ACE) levels would have been useful given the proposed importance of AngII in accentuating the T2DM risk in obese men, but these measurements have not been made. However, there are strong and consistent published results which demonstrate that ACE levels (and therefore AngII) in subjects with the ACE D allele are significantly higher than those with the I allele [4,5], and this effect of ACE I/D polymorphism remains the most likely direct mechanism.

The incidence of T2DM could possibly have been underestimated in the non-obese subjects as a whole, in view of general practitioners being more alert to the presence of T2DM in obese, rather than non-obese, patients. However, this is unlikely in this sample, given the thorough systematic review of patients’ notes, and it is worth noting that > 75% of cases with T2DM were from the non-obese group over the 15 years of follow-up. Even if this did take place, it is unlikely that it would affect the major conclusions of the studies, because it is implausible that any such underestimation of T2DM would occur by ACE (I/D) genotype. All of the 2489 subjects without T2DM are still alive, since they are ‘flagged’ with the Office of National Statistics and we receive all death certificates. At recruitment, no subjects were taking ACE inhibitors, ARBs and β-receptor blockers and, although a proportion of those who developed T2DM over follow-up may have been prescribed such medication, this is unlikely to have confounded the genetic effect on risk observed in the present study, but would rather have the effect of diluting it.

A further limitation is that the present study is underpowered to detect associations in which alleles have a small effect. Multiple testing (in this case for interaction and adjustments) may raise the probability that ‘chance’ is the explanation for the observed associations, and these results may therefore be false positives. A similar prospective study of T2DM was not available to us to try and replicate the findings. Re-analysis of a previously published case-control study showed a higher risk of T2DM in obese D allele carriers compared with II subjects, but the genotype–obesity interaction was not significant. There was, however, limited power (only 60% to detect the previously observed effect at the 5% significance level) to demonstrate such an effect size. Studies in which there are much larger numbers of cases and controls are necessary for firm conclusions.

In conclusion, this is the first prospective study to show that variation in the ACE gene may interact with BMI to increase the risk of T2DM. Prospective gene–association studies are better at teasing out gene–environment interactions than the case-control design, since the environment can be measured at baseline, and results are not confounded by survivor bias and retrospective patient recall of lifestyle factors [32]. Further replication is certainly required to confirm these findings. The combination of
the pro-inflammatory D allele and an already ‘at risk’ obese state may well underlie an impaired metabolic profile and a possible increased propensity to T2DM.

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REFERENCES


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