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# Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy

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**Summary** An insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene has repeatedly been shown to be associated with ischaemic heart disease, but the association of this genetic marker with diabetic microangiopathy is controversial. To assess the association of the genotypes with the development of diabetic nephropathy or retinopathy, we performed a meta-analysis of data from the literature, using Mantel-Haenszel method followed by the Breslow-Day test for assessing homogeneity among data. In a total of 4773 diabetic patients from 18 studies with ($n = 2495$) and without ($n = 2278$) renal complications, the D allele was significantly associated with diabetic nephropathy ($p < 0.0001$) in a dominant model (summary odds ratio 1.32, 95 % confidence interval: 1.15 to 1.51). There was no significant evidence against homogeneity of the odds ratios ($\chi^2 = 18.9$, 20 *df*; $p = 0.53$). The association was significant both in non-insulin-dependent ($p < 0.005$) and in insulin-dependent diabetes mellitus ($p < 0.05$). Likewise, in a total of 2010 diabetic patients with ($n = 1008$) and without ($n = 1002$) retinopathy, there was no association of the I/D polymorphism with diabetic retinopathy. These data suggest that the ACE I/D polymorphism affects the risk for diabetic nephropathy, but not for diabetic retinopathy. [Diabetologia (1998) 41: 47--53]

**Keywords** Angiotensin I-converting enzyme gene, I/D polymorphism, meta-analysis, diabetic nephropathy, diabetic retinopathy, genetic susceptibility.

Several lines of evidence from family and twin studies have strongly suggested that genetic factors are involved in the development of diabetic microangiopathy [1--3]. Several candidate genes have been investigated to elucidate genetic factor(s) responsible for the vascular complications, but little is known about the genetic basis of these complications [4, 5]. Elucidation of the genetic factors predisposing to chronic vascular complications in diabetes mellitus will permit identification of individuals genetically predisposed to the complications and, in turn, will allow us an effective intervention tailored to the specific underlying abnormalities.

The renin-angiotensin system regulates the systemic circulation and local haemodynamics, and also regulates cell growth and matrix production via its action on angiotensin II production. Angiotensin I-converting enzyme (ACE; EC 3.4.15.1) is not only a key enzyme in the renin-angiotensin system but also regulates kinin metabolism [6]. The plasma ACE level is under genetic control and is strongly associated with an insertion/deletion (I/D) polymorphism of the ACE gene, defined by the presence or absence of the 287 base-pair *Alu*-repetitive sequence in intron 16 [7]. Accordingly, the I/D polymorphism has been investigated as a strong candidate marker for genetic predisposition to diabetic vascular complications. Association studies of the ACE genotype with diabetic complications, however, have yielded conflicting results.

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*Abbreviations:* ACE, Angiotensin-converting enzyme; I/D, insertion/deletion; CI, confidence interval; *df,* degrees of freedom.

In the present study we investigated whether or not the ACE genotype is associated with diabetic microangiopathy by performing a meta-analysis of the data from the literature.

## Subjects and methods

*Identification of studies.* Medical reports based on clinical observations published in English after 1992, when the first report of the association of this genetic polymorphism with disease was published [8], were considered in this meta-analysis. In addition to identify studies, a search was performed on MEDLINE using the following keywords: (angiotensin converting enzyme or ACE) and (polymorphism or genotype or DD) and (diabetes mellitus or (insulin-dependent) IDDM or (non-insulin-dependent) NIDDM). In addition, complimentary searches in the references list of selected articles were performed. In each analysis, only studies with information on the genotype distribution according to the complications were included [9]. When diabetic complications according to the genotypes were reported for more than one subpopulation (for example, NIDDM and IDDM) in one study, each subpopulation was considered separately. In assessing risk for diabetic nephropathy, genotype data were divided into two separate groups, nephropathy group and control group. The nephropathy group consisted of patients with end-stage renal disease, macroalbuminuria, microalbuminuria (when urine albumin was measured quantitatively), and patients with proteinuria (when urine protein was qualitatively measured). The control group consisted of diabetic individuals defined as the control in each study. When patients were divided into more than two groups according to renal status, the nephropathy group consisted of those with microalbuminuria or more severe renal status. In another study, "decline group"" and "stable group"" defined originally in the manuscript were taken as the nephropathy group and the control group, respectively. As for diabetic retinopathy, genotype data were divided into two groups, retinopathy group and control group. The retinopathy group consisted of those showing retinal change, while the control group was defined as those having no signs of retinopathy with some restrictions as defined in each paper.

*Statistical methods.* For calculation of summary odds ratio according to the genotype groups, we adopted a fixed model using Mantel-Haenszel method [10] followed by the Breslow-Day test for assessing the heterogeneity of the strength of the association [11]. The 95% confidence interval (95% CI) was also calculated.

## Results

*Meta-analysis of nephropathy.* In total, 50 studies were retrieved by the search. With respect to the association of ACE genotype with nephropathy, a total of 18 studies with sufficient information have been published to date [12--29], and data on 21 subgroups containing diabetic subjects with ($n$ = 2495) and without ($n$ = 2278) nephropathy were available (Table 1). In most studies, the genotype distribution in the control group was consistent with Hardy-Weinberg equilibrium, except for two study groups (Table 2)

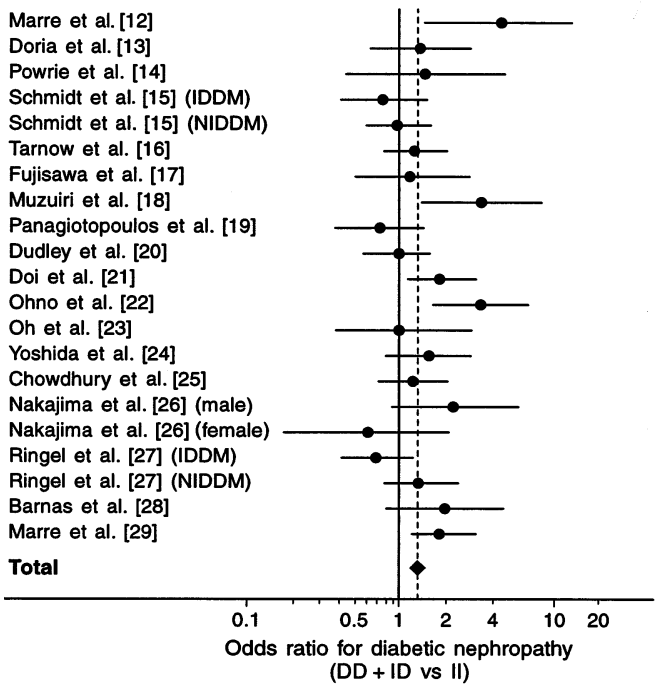


**Fig. 1.** Estimates of odds ratio for diabetic nephropathy according to insertion/deletion polymorphism of ACE gene. Values more than 1 imply an increased odds ratio for diabetic nephropathy associated with the DD + ID genotype (vs II genotype). 95% CIs expressed by bars (for each group) and ◆ (for all studies combined). Broken vertical line represents summary odds ratio of the total pooled data. Reference number given in parenthesis

[16, 27]. By the Mantel-Haenszel method, the D allele was significantly associated with diabetic nephropathy ($p < 0.0001$) in a dominant model (DD + ID vs II). The summary odds ratio for diabetic nephropathy was 1.32 (95% confidence interval [CI]: 1.15 to 1.51) (Fig. 1), indicating that the ratio of patients with/without nephropathy is 1.32 times higher in subjects with the D allele than in those without. In this model, there was no evidence for lack of homogeneity of the difference ($\chi^2 = 18.9$ with 20 *df*, $p = 0.53$), indicating that the results are compatible with a homogeneous association over the groups. By using the DerSimonian-Laird method, a random effect model, instead of the fixed effect model, the association of the D allele with nephropathy was also found to be significant (summary odds ratio: 1.25, 95% CI: 1.03 to 1.52). Separate analysis of IDDM and NIDDM subgroups was also performed by independently comparing the association of ACE genotype with renal complications. The association of ACE genotype with nephropathy was significant both in patients with NIDDM ($p < 0.005$, summary odds ratio: 1.36, 95% CI: 1.22 to 1.64) and in patients with IDDM ($p < 0.05$, summary odds ratio: 1.28, 95% CI: 1.05 to 1.57), where the test for heterogeneity in each group was not significant ($\chi^2 = 16.1$ with 10 *df*, $p < 0.09$ and

**Table 1.** Characteristics of studies reporting ACE genotype distribution in diabetic patients with nephropathy and control subjects

| Study | Type of diabetes | Setting |
|---|---|---|
| Marre et al. [12] | IDDM | France, single centre, *Cases:* Incipient or established nephropathy (UAE ≥ 30 mg/24 h), *Control:* age, sex duration retinopathy-matched normotensive normoalbuminuric (UAE < 30 mg/24 h) patients from hospital |
| Doria et al. [13] | IDDM | USA, single centre, duration 15 to 20 years, *Cases:* micro- (30 < AER < 250 μg/min) or macroalbuminuric (AER > 250 μg/min) patients or patients with ESRD, *Control:* normoalbuminuric (AER < 30 μg/min) patients |
| Powrie et al. [14] | IDDM | UK, outpatient clinics, duration 5 to 20 years, *Cases:* developed microalbuminuria (albumin/creatinine ratio > 3 mg/mmol) or overt renal failure over 10 years follow up, *Control:* remaining normoalbuminuric (albumin/creatinine ratio < 3) during the follow up |
| Schmidt et al. [15] | IDDM + NIDDM | German and Polish, 5 German outpatient clinics and 1 Polish clinic and 5 dialysis centres in Germany, duration > 10 years, *Cases:* microalbuminuria (UAE > 30 mg/24 h or > 250 μg/min) or more severe, *Controls:* normoalbuminuric patients |
| Tarnow et al. [16] | IDDM | Denmark, single outpatient clinic, *Cases:* persistent albuminuria of 300 mg/24 h with retinopathy, *Control:* age, sex, duration-matched persistent normoalbuminuric patients recruited from the outpatient clinic |
| Fujisawa et al. [17] | NIDDM | Japan, 2 hospitals, *Cases:* persistent proteinuria or ESRD, *Controls:* without proteinuria, duration > 10 years |
| Mizuiri et al. [18] | NIDDM | Japan, 1 hospital, duration > 10 years, *Cases:* micro- (AER: 20--200 μg/min) or macroalbuminuric (> 200 μg/min) patients, *Controls:* normoalbuminuric (< 20 μg/min) patients |
| Panagiotopoulos et al. [19] | NIDDM | Australia, 1 hospital, *Cases:* micro- (20--200 μg/min) or macroalbuminuric (> 200 μg/min) patients, *Controls:* normoalbuminuric (< 20 μg/min) patients |
| Dudley et al. [20] | NIDDM | UK, selected among 4860 patients, *Cases:* patients with urine in top tertile of the median UAE, *Controls:* age, sex, age-at-diagnosis, $HbA_{1c}$ and triglyceride-matched patients |
| Doi et al. [21] | NIDDM | Japan, hospital outpatient clinic, duration ≥ 10 years, *Cases:* UAI > 30 mg/g creatinine or ESRD, *Controls:* UAI < 30 mg/g creatinine |
| Ohno et al. [22] | NIDDM | Japan, hospital outpatient clinic, duration ≥ 5 years, *Cases:* micro- (10 < UAI < 200 mg/g creatinine) and macroalbuminuric (UAI > 200 mg/g creatinine) or ESRD, *Controls:* normoalbuminuric (UAI < 30 mg/g creatinine) patients |
| Oh et al. [23] | IDDM | Korea, Duration > 5 years, *Cases:* patients with microalbuminuria (20 < AER < 200 μg/min), overt proteinuria (urinary protein > 500 mg/24 h) or ESRD, *Controls:* normoalbuminuric (AER < 20 μg/min) patients |
| Yoshida et al. [24] | NIDDM | Japan, hospital outpatient clinic, duration ≥ 10 years, *Cases:* declining renal function as defined in the paper, *Controls:* with stable renal function |
| Chowdhury et al. [25] | IDDM | UK, multi-centre, Caucasian, *Cases:* patients with persistent Albustix positive, duration > 5 years, *Controls:* patients without proteinuria, and duration ≥ 15 years |
| Nakajima et al. [26] | NIDDM | Japan, hospital outpatient clinic, duration ≥ 5 years, *Cases:* patients with microalbuminuria (UAI > 30 mg/g creatinine) persistent proteinuria, *Controls:* normoalbuminuric (UAI < 30 mg/g creatinine) patients |
| Ringel et al. [27] | IDDM + NIDDM | Germany, Caucasian, 1 diabetes clinic and 4 dialysis centres, *Cases:* albumin excretion > 30 mg/24 h or > 20 μg/min, or ESRD, *Controls:* not specified |
| Barnas et al. [28] | IDDM | Austria, outpatient clinic, *Cases:* microalbuminuria of 30--300 mg/day or overt albuminuria or ESRD, *Controls:* not specified |
| Marre et al. [29] | IDDM | France and Belgium, 17 diabetic clinics, past or present proliferative retinopathy, *Cases:* patients with microalbuminuria (urinary albumin 30-300 mg/24 h, 20--200 μg/min, or 20--200 μg/l) or established and advanced nephropathy, *Controls:* urinary albumin < 30 mg/24 h, < 20 μg/min or 20 mg/l |

UAE, urinary albumin excretion; AER, albumin excretion ratio; ESRD, end-stage renal disease; UAI, urinary albumin index

$\chi^2 = 2.2$ with 9 *df*, $p > 0.9$, respectively). When the nephropathy group was limited to patients with macroalbuminuria or more severe nephropathy, the association of the ACE genotype with nephropathy was also significant ($p < 0.005$, summary odds ratio: 1.33, 95% CI: 1.10 to 1.62) [16, 17, 19, 21, 22, 24--26, 29]. As the ethnic heterogeneity of the ACE polymorphism is well known, ethnic groups were separately analysed. The association was significant both in Asian populations ($p < 0.00005$, summary odds ratio: 1.79, 95% CI: 1.37 to 2.33: 8 subgroups) [17, 18, 21--24, 26], and in Caucasian populations ($p < 0.05$, summary odds ratio: 1.18, 95% CI: 1.00 to 1.39: 13 subgroups [12--16, 19, 20, 25, 27--29], respectively.

*Meta-analysis of retinopathy.* As for the association of ACE genotype with diabetic retinopathy, data on diabetic individuals with ($n = 1008$) and without ($n =$

**Table 2.** Reported distribution of genotypes of AGE gene in diabetic patients with nephropathy and control subjects

| Study | Genotype | | | | | | (HW)[a] | Odds ratio[b] |
|---|---|---|---|---|---|---|---|---|
| | Nephropathy | | | Control Subjects | | | | |
| | DD | ID | II | DD | ID | II | | |
| Marre et al. [12] | 23 | 35 | 4 | 19 | 28 | 15 | 0.46 | 4.63 |
| Doria et al. [13] | 24 | 35 | 15 | 16 | 41 | 20 | 0.55 | 1.38 |
| Powrie et al. [14] | 7 | 8 | 4 | 24 | 37 | 24 | 0.23 | 1.48 |
| Schmidt et al. [15] (IDDM) | 52 | 38 | 62 | 55 | 55 | 23 | 0.16 | 0.78 |
| (NIDDM) | 101 | 105 | 41 | 83 | 91 | 34 | 0.29 | 0.98 |
| Tarnow et al. [16] | 63 | 95 | 40 | 67 | 77 | 46 | 0.013 | 1.26 |
| Fujisawa et al. [17] | 7 | 23 | 24 | 6 | 12 | 17 | 0.15 | 1.18 |
| Mizuiri et al. [18] | 19 | 51 | 11 | 9 | 11 | 11 | 0.11 | 3.45 |
| Panagiotopoulos et al. [19] | 37 | 38 | 25 | 30 | 50 | 20 | 0.92 | 0.75 |
| Dudley et al. [20] | 47 | 85 | 31 | 70 | 148 | 49 | 0.059 | 0.96 |
| Doi et al. [21] | 29 | 85 | 50 | 12 | 56 | 56 | 0.71 | 1.88 |
| Ohno et al. [22] | 15 | 38 | 26 | 5 | 15 | 33 | 0.12 | 3.36 |
| Oh et al. [23] | 10 | 9 | 12 | 7 | 10 | 11 | 0.15 | 1.02 |
| Yoshida et al. [24] | 19 | 28 | 25 | 7 | 46 | 43 | 0.26 | 1.53 |
| Chowdhury et al. [25] | 78 | 124 | 40 | 55 | 79 | 32 | 0.70 | 1.21 |
| Nakajima et al. [26] (male) | 10 | 35 | 20 | 1 | 11 | 12 | 0.43 | 2.25 |
| (female) | 4 | 15 | 17 | 3 | 8 | 6 | 0.91 | 0.61 |
| Ringel et al. [27] (IDDM) | 35 | 68 | 31 | 57 | 130 | 39 | 0.018 | 0.69 |
| (NIDDM) | 44 | 84 | 33 | 35 | 69 | 36 | 0.87 | 1.34 |
| Barnas et al. [28] | 17 | 35 | 11 | 7 | 34 | 18 | 0.14 | 2.08 |
| Marre et al. [29] | 119 | 168 | 50 | 48 | 69 | 40 | 0.14 | 1.96 |

Data are number of patients
[a] Probability value for Hardy-Weinberg equilibrium in control group; [b] Odds ratio for DD + ID vs II genotypes

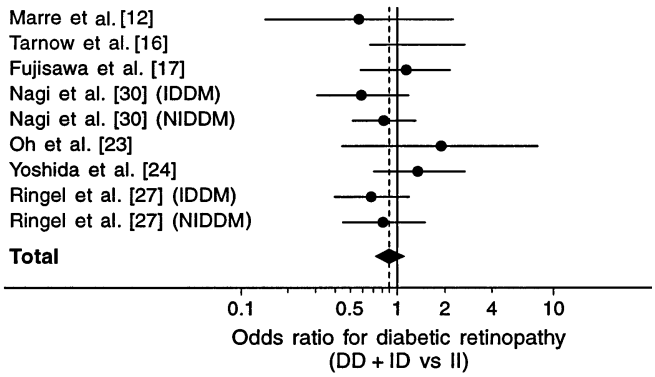


**Fig. 2.** Estimates of odds ratio for diabetic retinopathy according to insertion/deletion polymorphism of angiotensin-converting enzyme gene. 95% CIs expressed by bars (for each group) and ◆ (for all studies combined). Broken vertical line represents summary odds ratio of the total pooled data. Reference number given in parenthesis

1002) retinopathy were available from seven studies [12, 16, 17, 23, 24, 27, 30]. The summary odds ratio of D allele in a dominant model (DD + ID vs II) for diabetic retinopathy was 0.91 (95% CI: 0.73 to 1.13; eight subgroups) (Fig. 2), with no evidence of heterogeneity among the nine subgroups ($\chi^2 = 2.36$, 8 *df;* $p > 0.9$).

## Discussion

This study represents the first application of meta-analysis to assess the risk for diabetic microangiopathy in relation to the I/D polymorphism of the ACE gene. Based on data from more than 5000 diabetic individuals in total, it is concluded that this polymorphism is associated with diabetic nephropathy but not with diabetic retinopathy. In addition, the association among the diverse population groups exhibited a relatively similar strength despite the different genetic (including distribution of ACE genotypes) and environmental backgrounds. Therefore, the ACE I/D polymorphism is suggested to affect the risk for diabetic nephropathy, but not for retinopathy, in a universal manner.

The calculated odds ratio of 1.32 presents us with two important pieces of information. First, assuming the $\lambda$s value, the degree of familial clustering of a disease, to be 2.17 for diabetic nephropathy based on the data on Caucasians [3, 31], it can be calculated from the estimation of odds ratio of 1.32 (95% CI: 1.15 to 1.51) in D-allele carriers that the ACE locus contributes 28% (14--43%) to the familial clustering of nephropathy. Second, since D-allele carriers comprise 50--85% of each ethnic population and the D allele is universally associated with renal complica-

tions, if the ratio of patients with/without nephropathy in D-allele carriers, were reduced from 1.32 times that in non-carriers to the non-carriers' own baseline, one would expect a 14--21 % reduction of renal insufficiency due to diabetic nephropathy throughout the world. The use of ACE inhibitors appears to be a theoretical intervention tailored to the underlying specific abnormalities, because it is possible that the D allele exerts its effect via elevated ACE level. Indeed, in the management of individuals with proteinuria including diabetic subjects, the DD genotype was reported to be associated with administration of an ACE inhibitor having an antiproteinuric effect [32].

As shown in the current study, meta-analysis turned out to be a useful strategy for elucidating genetic factors in multifactorial disorders, such as diabetic complications. Due to the nature of multifactorial diseases, the impact of one genetic component on the development of the disease may be easily masked by other genetic and environmental factors. From this point of view, a meta-analysis is a powerful strategy, because: 1) it potentially investigates a large number of individuals; 2) it can estimate the impact of a genetic factor on the risk for the disease; and 3) it also can assess whether the association is common, or differs depending upon some specific clinical or genetic background feature [33]. Therefore, meta-analysis is potentially applicable for assessing the contribution of genetic factors not only to diabetic complications, but also to complex traits in general.

The development of diabetic microangiopathy is suggested to be, at least in part, genetically determined. Little is known, however, about the genetic factors responsible for the susceptibility. Furthermore, it is not clear whether genetic factors contributing to retinopathy and nephropathy are the same or different. In contrast to the significant association of the I/D polymorphism with diabetic nephropathy, our current meta-analysis as well as published reports did not support an association of the I/D polymorphism with diabetic retinopathy, suggesting that the ACE locus is unlikely to contribute to susceptibility to retinopathy. Alternatively, since the I/D polymorphism is associated with progression of other renal diseases [34, 35], the ACE locus may determine the susceptibility to renal damage in general, and, in coexistence with diabetes, it may act to increase the risk for diabetic nephropathy. The observed difference in the associations between retinopathy and nephropathy suggests that the genetic factors responsible for the two types of microangiopathy are different. Other possible interpretations derive from the difficulty in specifying an adequate endpoint (proliferative vs non-proliferative retinopathy).

An important unresolved issue concerning the effect of the D allele on disease susceptibility is the genetic mechanism; i. e. whether the effect is recessive, codominant, or dominant. The increased level of plasma ACE associated with the I/D polymorphism is suggested to be a codominant trait, with a low level in II genotype, intermediate in ID and highest in DD. As for the risk of the D allele for large vessel disease, taking into account a reported meta-analysis examining coronary heart disease [33] and three earlier studies investigating myocardial infarction in patients with NIDDM [17, 36, 37], the effect of the genotype may well be recessive (DD vs ID + II). In contrast, our present meta-analysis showed that the effect of the D allele on susceptibility to nephropathy is likely to be dominant. This is because the summary odds ratio calculated in a recessive model (1.20, 95 % CI: 1.06 to 1.37) was not higher than that in a dominant model (1.32), and the recessive model lessened homogeneity among groups ($p = 0.31$ in a recessive vs $p = 0.53$ in a dominant model, Breslow-Day test). Taken together, assuming that I/D polymorphism is involved in disease susceptibility through its effect on ACE level, it may be possible that the threshold for renal complications, if it exists, may be different from, and probably lower than, that for macroangiopathy.

Since the mechanisms of hyperglycaemia-induced tissue damage are likely to be common between NIDDM and IDDM, the genetic factors contributing to diabetic microangiopathy seem to be the same. However, there is little evidence supporting this hypothesis. Based on our separate analysis of NIDDM and IDDM, the association of the ACE genotype with diabetic nephropathy was found to be significant both in patients with NIDDM and in patients with IDDM. These data suggest a common genetic contribution, at least regarding the ACE I/D polymorphism, to diabetic complications in NIDDM and IDDM.

We are aware that these analyses have several limitations; namely limited number and size of available studies and the effect of publication bias, which may cause overestimation of the risk. Therefore, the results must be cautiously interpreted. Some of the odds ratios from the studies, in which originally no association was reported between diabetic nephropathy and ACE polymorphism, were more than 1.0 (Fig. 1). In fact, in such studies, the bars for 95 % CIs of the odds ratios cross the odds ratio = 1 line. Many of them might not have enough power to detect the association. To confirm the hypothesis of the contribution of D allele to the genetic predisposition to diabetic nephropathy, a larger scale study is necessary; i. e. about 1200 cases, and a similar number of control diabetic individuals needs to be analysed to obtain a statistical power of 80 % to detect a difference at $p = 0.05$, given the effect on risk for nephropathy as 1.32 and II genotype frequency as 0.25 [34]. If the effect is smaller, as discussed above, a much larger size would be necessary. Thus, our meta-analysis also pro-

vides information to help plan the sample size for future studies [38].

It is possible that heterogeneous groups were included in the nephropathy group. The additional analysis in which the nephropathy group was limited to patients with macroalbuminuria or more severe disease also showed that the association of ACE genotype with nephropathy was significant ($p < 0.005$). However, this approach does not distinguish whether the genotype increases the susceptibility to diabetic nephropathy or accelerates progression of existing nephropathy (or both). When one study [24], in which the patients without progression of nephropathy were treated as "stable"", was excluded from the current meta-analysis, the summary odds ratio was 1.31 (95% CI: 1.14 to 1.51), where the test for heterogeneity was not significant ($p = 0.47$).

In summary, our present meta-analysis demonstrated that the I/D polymorphism was associated with diabetic nephropathy, but not with retinopathy, across different population groups, suggesting that the ACE I/D polymorphism affects the universal risk for diabetic nephropathy, but not for retinopathy.

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