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Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects

Abstract *Aims/hypothesis:* The *ACE* insertion/deletion polymorphism has been examined for association with diabetic nephropathy over the past decade with conflicting results. To clarify this situation, we conducted a comprehensive meta-analysis encompassing all relevant studies that were published between 1994 and 2004 and investigated this potential genetic association. *Methods:* A total of 14,727 subjects from 47 studies was included in this meta-analysis. Cases (n=8,663) were type 1 or 2 diabetic subjects with incipient (microalbuminuria) or advanced diabetic nephropathy (proteinuria, chronic renal failure, end-stage renal disease). Control subjects (n=6,064) were predominantly normoalbuminuric. *Results:* No obvious publication bias was detected. Using a minimal-case definition based on incipient diabetic nephropathy, subjects with the II genotype had a 22% lower risk of diabetic nephropathy than carriers of the D allele (pooled odds ratio [OR]=0.78, 95% CI=0.69–0.88). While there was a reduced risk of diabetic nephropathy associated with the II genotype among Caucasians with either type 1 or type 2 diabetes, the association was most marked among type 2 diabetic Asians (Chinese, Japanese, Koreans) (OR=0.65, 95% CI=0. 51–0.83). This OR is significantly different from the OR of 0.90 (95% CI= 0.78–1.04) that was obtained for type 2 diabetic Caucasians (*p*=0.019). Using a stricter case definition based on advanced diabetic nephropathy, a comparable risk reduction of 24–32% was observed among the three subgroups, although statistical significance was reached only among Asians. *Conclusions/*

interpretation: The results of our meta-analysis support a genetic association of the *ACE* Ins/Del polymorphism with diabetic nephropathy. These findings may have implications for the management of diabetic nephropathy using ACE inhibitors especially among type 2 diabetic Asians.

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Introduction

Diabetic nephropathy is the most serious complication of diabetes mellitus and affects approximately a third of diabetic patients. Importantly, it is the leading cause of end-stage renal disease requiring dialysis or transplantation in developed countries [1] as well as in rapidly developing countries in Asia [2]. Besides good glycaemic control, measures such as the attenuation of the renin–angiotensin system using ACE inhibitors are important in the clinical management of this complication [3]. However, the efficacy of ACE inhibitors in treating diabetic nephropathy can be influenced by genetic variation at the *ACE* gene locus [4, 5]. Specifically, a polymorphism termed Ins/Del, which results from the insertion/deletion of a 287 bp Alu sequence in intron 16, accounts for half the variance of serum enzyme levels with individuals homozygous for the insertion allele (II genotype) having significantly lower levels than carriers of the deletion allele (ID and DD genotypes) [6].

Studies of familial clustering have consistently demonstrated that genetic susceptibility plays an important role in diabetic nephropathy [7]. Regarding *ACE*, investigators have sought to determine if the Ins/Del polymorphism acts as a genetic risk factor for diabetic nephropathy, beginning with the initial study by Marre et al in 1994, who reported a protective effect of the II genotype on the development of this complication [8]. However, meta-analyses pub-

lished in 1998 did not support this conclusion among Caucasians with either type 1 or type 2 diabetes, although the II genotype appeared protective among type 2 diabetic Asians of Japanese and Korean origin [9, 10]. While genetic susceptibility linked to the *ACE* locus may be population-specific, an important caveat is that these earlier reviews were conducted on just around 20 studies and comprised about 5,000 subjects each. This limited data did not allow firm conclusions from such detailed subgroup analyses [9, 10]. A third meta-analysis did not include the relevant subgroup analyses [11].

Since 1998, there has been a considerable surge in the number of reports on the association of the *ACE* Ins/Del polymorphism with diabetic nephropathy. Indeed, our present literature search indicated that the number of such publications has doubled at least since the previous meta-analyses. In light of this significant development, we conducted a new meta-analysis based on 47 studies published during the decade starting from 1994 and which comprises 14,727 diabetic subjects.

Subjects, materials and methods

Identification of studies We identified studies (case-control, cross-sectional or cohort study designs) that examined the association of the *ACE* Ins/Del polymorphism with diabetic nephropathy and were published from 1994 to March 2004, beginning with the first report by Marre et al [8]. Studies were identified by searching the PubMed database (http://www.ncbi.nlm.nih.gov/PubMed/) using the keywords 'angiotensin converting enzyme', 'diabetes', 'diabetic nephropathy', 'end-stage renal disease' and 'polymorphism', with keywords used singly and in various combinations. All studies included in previous meta-analyses were also considered [9–11]. The reference lists from all identified studies were also scrutinised to search for additional reports. Studies were considered, if they provided sufficient information to allow a comparison of the *ACE* Ins/Del genotype distribution between cases and control subjects. Cases were type 1 or 2 diabetic subjects with incipient (microalbuminuria) or advanced diabetic nephropathy (proteinuria/macroalbuminuria, chronic renal failure, end-stage renal disease), while control subjects were defined predominantly on the basis of normoalbuminuria (Table 1).

Selection of studies for inclusion Fifty-eight studies arising from 57 individual publications were identified for possible inclusion in this meta-analysis [8, 12–67]. Of these, nine studies appeared to overlap with more recent publications and were excluded to avoid partial or complete duplication of data [8, 18, 35, 42, 48, 53, 57, 60, 67]. A study by Gutierrez et al. [28] was excluded since it reported only allele, but not genotype distributions. A small study (*n*=30) was excluded as the published data did not allow an estimation of the study-specific OR or its contribution to the pooled OR, since there were empty cells in the reported genotype distributions [62]. The remaining 47 studies ful-

filled the criteria for inclusion in this review. Together, these studies comprised 8,663 cases and 6,064 control subjects, yielding a total of 14,727 subjects.

Data extraction The following information was extracted from the selected studies: names of first and corresponding authors, year of publication, journal citation, country in which the study was conducted, race of study population, study design, genotyping method, type of diabetes, case/control definitions and genotype distribution of the Ins/Del.

Statistical analyses Funnel plots of the effect estimate based on log–odds ratio were plotted against the reciprocal of its standard error to evaluate the possibility of publication bias [68]. The chi square test for goodness-of-fit was used to determine if there was significant deviation from Hardy–Weinberg equilibrium among the control subjects in the individual studies. The magnitude of the genetic association between the *ACE* Ins/Del polymorphism and diabetic nephropathy was obtained by calculating the odds ratio (OR) and its associated 95% CI. A random effects model was employed to summarise the data. This model assumes that the 47 studies selected for inclusion in the review are a random sample from the larger population of such studies, with each study having its own underlying effect size. Under this model, it is assumed that there is a mean population effect size, about which the study-specific ORs vary. As the random effects model properly takes into account the inter-study heterogeneity such as differences in study design (case-control, cross-sectional and cohort studies) and case definitions for diabetic nephropathy, it provides a more conservative evaluation of the significance of the association than one based on fixed effects [69]. Data from the small number of cohort studies were included in the meta-analyses (Table 1); cases and control subjects were defined according to patient phenotypes after the duration of follow-up specified for each study. The stratification of studies by type of diabetes was subsequently considered. Subgroup analysis of data for type 1 diabetic subjects was restricted to Caucasians since these represented the majority (75%) of such studies. In the case of type 2 diabetic subjects, we analysed the data for Caucasians and Asians separately. The Asian subgroup consisted of Chinese, Japanese and Korean subjects from 16 studies. The risk of diabetic nephropathy attributable to carriage of the D allele was calculated using the formula $\hat{\lambda} = \frac{ad-bc}{d(a+b)}$ where a and c are the number of cases and control subjects who are carriers of the D allele, while b and d are cases and control subjects homozygous for the I allele [70].

Results

Studies selected for inclusion Forty-seven studies, comprising 8,663 cases and 6,064 controls, fulfilled the criteria for inclusion in this review (Table 1). Twenty studies (16

Table 1 Summary of the 47 studies included in the meta-analysis

First author	Ref.	Year	Race	Design	Diabetes	Case definition (renal status)	Control definition (renal status)	Case genotypes (n) DD	ID	II	Control genotypes (n) DD	ID	II
Doria	[12]	1994	Caucasian	CC	Type 1	Micro, Prot, ESRD	Normo	24	35	15	16	41	20
Powrie	[13]	1994	Caucasian	CH	Type 1	Micro	Normo after 10 years follow-up	7	8	4	24	37	24
Dudley	[14]	1995	Caucasian	CC	Type 2	Top tertile of albuminuria	Excluding top tertile	47	85	31	70	148	49
Fujisawa	[15]	1995	Asian Japanese	CC	Type 2	Prot, HD	No Prot with DM≥10 years	7	23	24	6	12	17
Mizuiri	[16]	1995	Asian Japanese	CS	Type 2	Micro, Prot, CRF	Normo, with DM≥10 years	19	50	11	9	11	11
Panagiotopoulos	[17]	1995	Caucasian	CS	Type 2	Micro, Prot	Normo	15	25	10	42	44	29
Schmidt	[18]	1995	Caucasian	CC	Type 1	Micro at least	Normo	52	38	24	55	55	23
Tarnow	[19]	1995	Caucasian	CC	Type 1	Prot	Normo	63	95	40	67	77	46
Rabensteiner	[20]	1995	Caucasian	CC	Type 1	Micro, Prot	Normo	16	39	9	8	33	15
Chowdhury	[21]	1996	Caucasian	CC	Type 1	Prot	No Prot with DM>15 years	78	124	40	55	79	32
Doi	[22]	1996	Asian Japanese	CC	Type 2	Micro, Prot, CRF, HD	Normo with DM<10 years	29	85	50	12	56	56
Nakajima	[23]	1996	Asian Japanese	CS	Type 2	Micro, Prot	Normo	14	50	37	4	19	18
Oh	[24]	1996	Asian Korean	CS	Type 1	Micro, Prot, ESRD	Normo	10	9	12	7	10	11
Ohno	[25]	1996	Asian Japanese	CS	Type 2	Micro, Prot,	Normo	15	38	26	5	15	33
Yoshida	[26]	1996	Asian Japanese	CS	Type 2	CRF, ESRD	Stable renal function	19	28	25	7	46	43
Barnas	[27]	1997	Caucasian	CC	Type 1	Micro, Prot, CRF, HD	Normo with DM>20 years	14	27	9	4	21	15
Hibberd	[29]	1997	Caucasian	CC	Type 1	Prot	Normo with DM≥20 years	21	42	9	36	43	7
Jeffers	[30]	1997	Caucasians	CS	Type 2	Prot	No Prot	23	20	7	139	218	102
Marre	[31]	1997	Caucasian	CS	Type 1	Micro, Prot, CRF, ESRD	Normo	119	168	50	48	69	40
Ringel	[33]	1997	Caucasian	CS	Type 1	Micro, ESRD	Normo	35	68	31	57	130	39
Ringel	[33]	1997	Caucasian	CS	Type 2	Micro, ESRD	Normo	44	84	33	35	69	36
Demurov	[34]	1997	Caucasian	CC	Type 1	Prot	Normo	24	29	3	24	32	20
Schmidt	[35]	1997	Caucasian	CC	Type 2	Micro at least, ESRD	Normo with DM≥10 years	121	129	61	131	154	62
Pfohl	[32]	1998	Caucasian	CS	Type 1	Micro, Prot, HD	Normo	17	15	8	15	18	7
Freire	[37]	1998	Jews	CS	Type 1	Micro, Prot	Normo with DM≥10 years	33	32	12	34	45	10
Grzeszczak	[38]	1998	Caucasian	CC	Type 2	Micro, Prot, CRF	Normo with DM>10 years	129	230	103	73	118	63
Hanyu	[39]	1998	Asian Japanese	CS	Type 2	Micro at least	Normo	4	13	7	2	5	14
Huang	[40]	1998	Caucasian	CH	Type 2	Micro, Prot	Normo after 9 years follow-up	11	16	2	20	25	9
Wu	[41]	1998	Asian Chinese	CC	Type 2	Diabetic nephropathy not explicitly defined	No diabetic nephropathy	12	18	21	1	11	6
Bouhanick	[43]	1999	Caucasian	CS	Type 1	Micro	Normo	4	5	4	19	34	10
De Cosmo	[44]	1999	Caucasian	CC	Type 1	Micro, Macro	Normo with DM>15 years	73	79	23	65	53	18

Table 1 (continued)

| First author	Ref.	Year	Race	Design	Diabetes	Case definition (renal status)	Control definition (renal status)	Case genotypes (n)			Control genotypes (n)		
								DD	ID	II	DD	ID	II
Kuramoto	[45]	1999	Asian Japanese	CS	Type 2	Micro, Prot	Normo with DM>5 years	9	16	8	3	13	13
Miura	[46]	1999	Asian Japanese	CS	Type 1	Micro, Macro, CRF, ESRD	Normo with DM>10 years	13	49	36	10	58	35
Vleming	[49]	1999	Caucasian	CC	Type 1	ESRD	Normo with DM>15 years	39	24	16	26	34	22
Wong	[50]	1999	Asian Chinese	CS	Type 2	Prot, CRF, ESRD	Normo, micro with DM>10 years	7	30	43	12	40	36
Hsieh	[51]	2000	Asian Chinese	CC	Type 2	Prot, HD	No Prot	40	59	80	21	50	86
van Ittersum	[52]	2000	Caucasian	CS	Type 1	Micro, Prot	Normo	13	33	23	49	86	53
Araz	[54]	2001	Middle East	CS	Type 2	Micro, Macro	Normo	34	64	18	43	57	23
Azar	[55]	2001	Middle East	CC	Type 1	Micro, Prot, ESRD	Normo with DM>15 years	23	27	2	1	7	2
Gohda	[56]	2001	Asian Japanese	CC	Type 2	Micro, Prot, CRF, ESRD	Normo with DM>15 years	85	222	229	31	92	89
Taniwaki	[58]	2001	Asian Japanese	CS	Type 2	Micro, Prot, CRF	Normo	14	40	32	12	26	31
Viswanathan	[59]	2001	Asian South Indian	CC	Type 2	Prot	Normo	24	45	17	5	8	10
Fradin	[61]	2002	Caucasian	CS	Type 2	Micro, Macro	Normo	38	61	18	44	54	20
Lee	[63]	2002	Asian Chinese	CS	Type 2	Micro, advanced nephropathy	Normo	40	137	117	39	170	208
Ha	[64]	2003	Asian Korean	CS	Type 2	CRF, ESRD	Normo with DM>15 years	43	62	35	9	57	33
Hadjadj	[65]	2003	Caucasian	CC	Type 2	Micro, Macro	Normo	1,119	1,468	552	208	282	115
Okuno	[66]	2003	Asian Japanese	CH	Type 2	Micro	Normo after 9 years follow-up	3	8	1	5	12	21

CC case-control, *CS* cross-sectional, *CH* cohort, *Normo* normoalbuminuria, *Micro* microalbuminuria, *Prot* proteinuria, *Macro* macroalbuminuria, *CRF* chronic renal failure, *ESRD* end-stage renal disease, *HD* haemodialysis, *DM* diabetes mellitus

Caucasian, 4 non-Caucasian) involved type 1 diabetic subjects, while the remaining 27 studies were conducted on patients with type 2 diabetes (9 Caucasian, 16 Asian and 2 others). The 16 studies on type 1 diabetic Caucasians contributed 1,736 cases and 1,801 control subjects. Among the type 2 diabetic studies, studies involving Caucasians contributed 4,482 cases and 2,359 control subjects, while the Asian studies included 1,985 cases and 1,528 control subjects.

Publication bias and Hardy–Weinberg equilibrium The potential presence of publication bias was evaluated using a funnel plot of the estimate of log–odds ratio for the genotype II versus DD+ ID against the reciprocal of its standard error (Fig. 1). There was considerable scatter around the pooled log–odds ratio estimate when the reciprocal of the standard error was small (i.e. corresponded to small studies); this scattering converged as the reciprocal of the

standard error increased (i.e. corresponded to larger studies). The resulting symmetrical funnel shape was consistent with an absence of publication bias since small studies with negative results were as likely to be published (Fig. 1a). No obvious funnel asymmetry was observed when this plot was repeated for studies comprising Caucasians with type 1 or type 2 diabetes and for studies of Asians with type 2 diabetes alone (Fig. 1b–d). Of the 47 studies, only two had control groups whose genotype distributions did not conform to that expected under Hardy–Weinberg equilibrium [19, 51]. For completeness of this review, these studies were retained for the data analysis. Exclusion of these studies did not materially alter the conclusions of this review (data not shown).

Association of Ins/Del with diabetic nephropathy—estimation of pooled OR To evaluate the magnitude of the association between *ACE* Ins/Del and diabetic nephropathy, we

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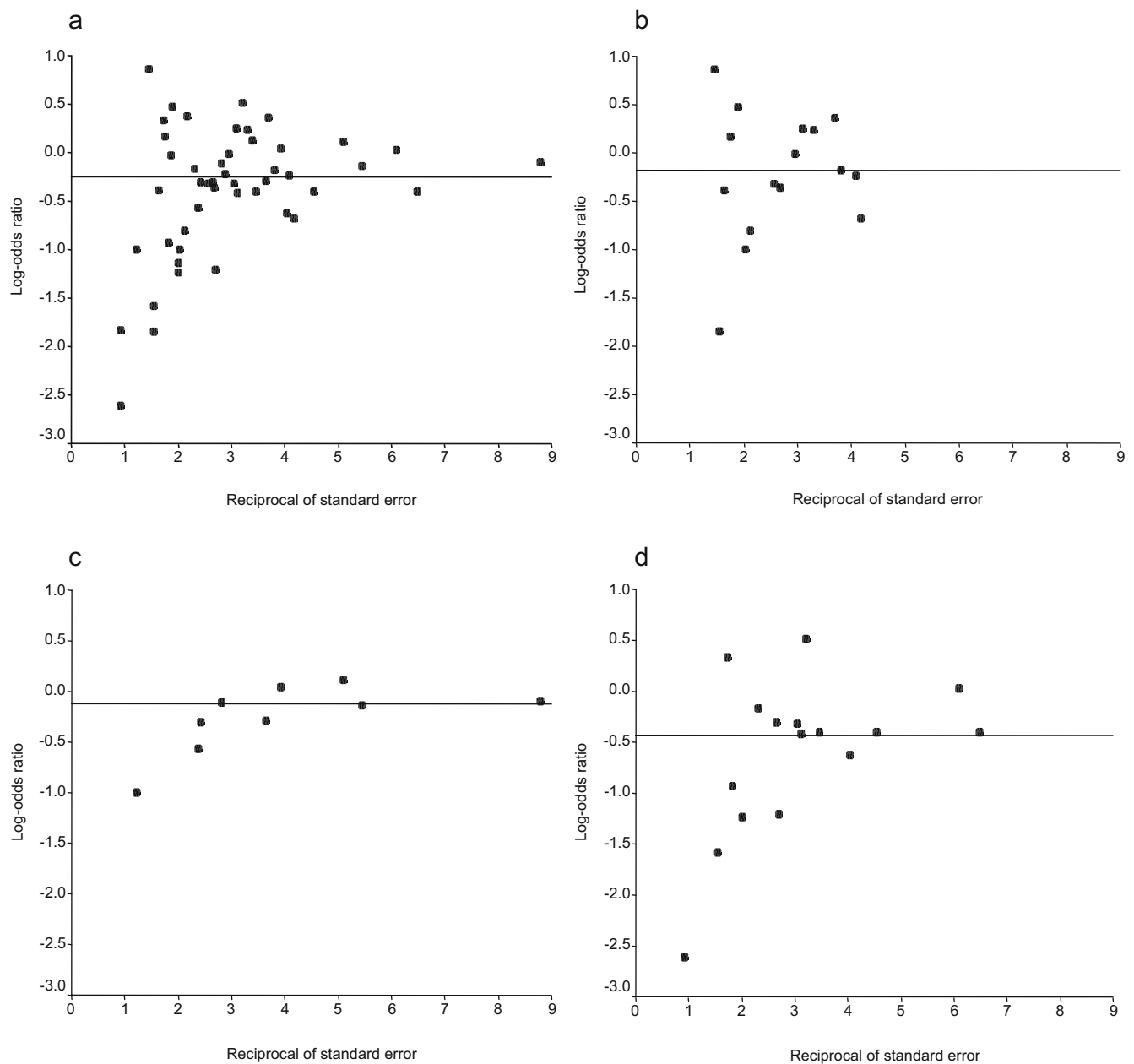
Fig. 1 Evaluation of publication bias using funnel plots. Plots are shown for all studies (**a**), and subgroups involving type 1 diabetic Caucasia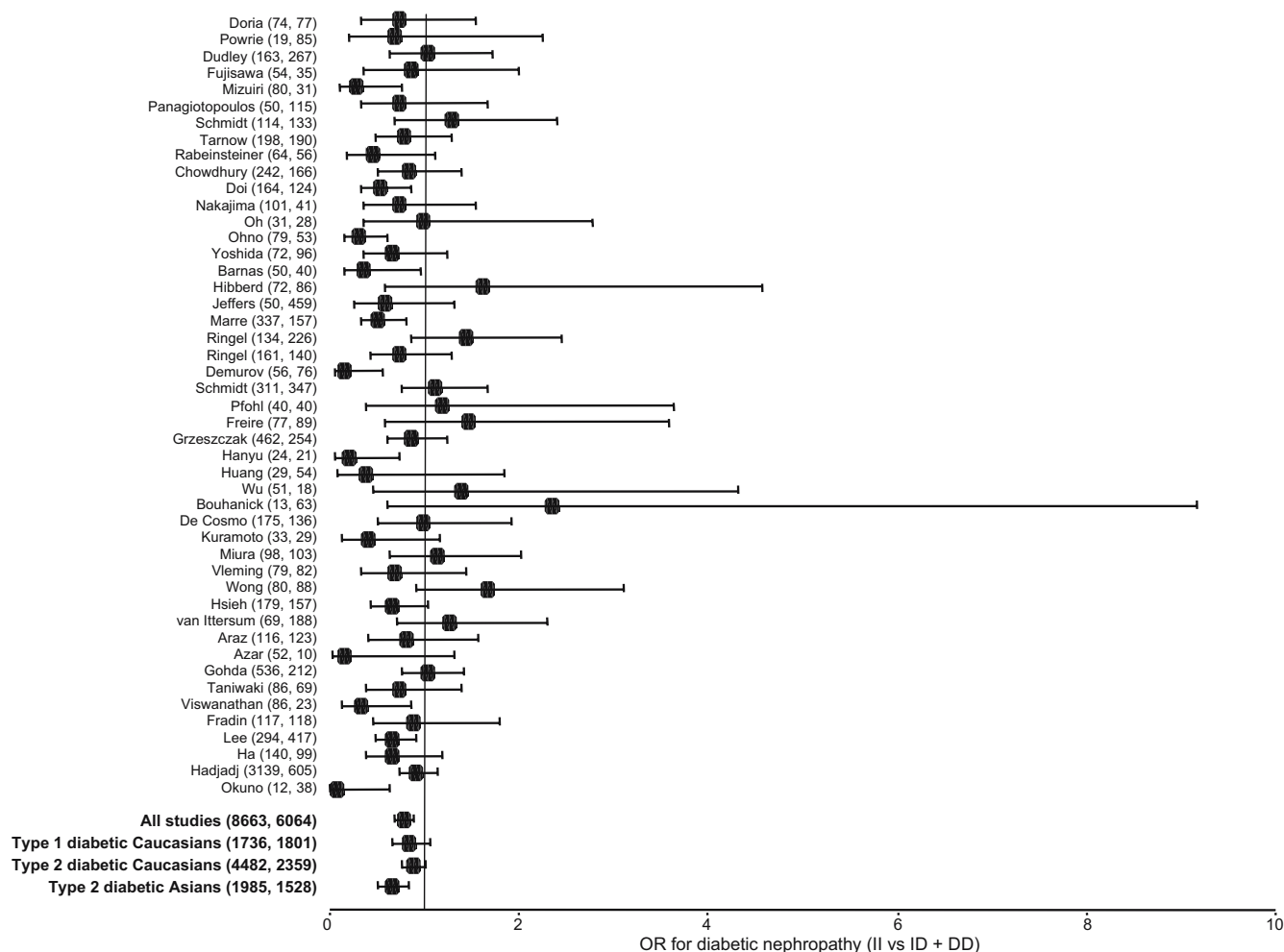ns (**b**), as well as Caucasians and Asians with type 2 diabetes (**c, d**). No significant funnel asymmetry was observed which could indicate publication bias

generated study-specific and pooled OR estimates for the comparison of the II genotype versus ID and DD genotypes separately. The ORs for II vs DD and II vs ID were 0.70 (95% CI 0.60–0.83) and 0.82 (95% CI 0.72–0.93) respectively. Thus, the risk of diabetic nephropathy associated with either DD or ID genotypes was of similar magnitude, and was consistent with II being the protective genotype. Based on all 47 studies, subjects with the II genotype had a 22% lower risk of diabetic nephropathy than carriers of the D allele (pooled OR= 0.78, 95% CI= 0.69–0.88) (Fig. 2).

We next refined our analysis by focussing on subgroups of studies comprising solely type 1 diabetic Caucasians, type 2 diabetic Caucasians and type 2 diabetic Asians.

While all three subgroup analyses showed that the II genotype is associated with a reduced risk of diabetic nephropathy, the association was most marked among Asians with type 2 diabetes. Specifically, Asians who were II homozygotes had a 35% reduction in risk of diabetic nephropathy compared to those carrying the D allele (OR= 0.65, 95% CI=0. 51–0.83). This 35% reduction in risk associated with the II genotype was significantly greater than the 10% observed among type 2 diabetic Caucasians (OR=0.90, 95% CI=0.78–1.04) (*p*=0.019), indicating that these subgroups were heterogeneous with regard to the association of the *ACE* Ins/Del with diabetic nephropathy. A small reduction in risk of diabetic nephropathy asso-

Fig. 2 Odds ratios (ORs) and the associated 95% CI comparing ACE II versus ID and DD genotypes combined. The graph shows individual and pooled estimates for all studies, and studies on type 1 diabetic Caucasians, as well as Caucasians and Asians with type 2 diabetes. Cases were defined on the minimal criterion of micro-albuminuria. The number of cases and control subjects for each study are in brackets

ciated with the II genotype also failed to reach statistical significance among the subgroup comprising 3,537 Caucasian patients with type 1 diabetes (OR=0.83, 95% CI= 0.65–1.07). Subgroup analysis was not performed for Asians with type 1 diabetes, since only two such studies were included in this review. The risk attributable to carriage of the D allele among type 2 diabetic Asians was modest at 20% (see Subjects, materials and methods).

A minimum diabetes duration, after which control subjects continued to be normoalbuminuric, was specified in several of the 47 studies (Table 1) and use of this subset of control subjects may reduce case misclassification. We therefore analysed the studies that employed such 'long-duration control subjects'. The OR for studies on type 1 diabetic Caucasians [13, 27, 29, 44, 49] was 0.78 (95% CI 0.51–1.21). For studies on type 2 diabetic Caucasians [35, 38, 40] and Asians [16, 45, 50, 56, 64, 66], the OR estimates were 0.95 (95% CI 0.71–1.27) and 0.65 (95% CI 0.37–1.15) respectively. These results were very similar to when all controls were considered, albeit with wider con-

fidence intervals as expected. Thus confining our analyses to studies with long-duration control subjects did not materially alter our findings.

The case definition employed in the published studies varied, ranging from incipient (indicated by microalbuminuria) to advanced diabetic nephropathy (proteinuria, chronic renal failure, end-stage renal disease). Thus, we repeated the meta-analyses based on 12 reports that had defined cases solely on the presence of advanced diabetic nephropathy [15, 19, 21, 26, 29, 30, 34, 49–51, 59, 64]. This was supplemented with data from 12 additional studies, which had separately furnished the genotype distributions of such advanced cases in their reports [22, 23, 25, 31, 35, 38, 46, 52, 54, 56, 58, 61] (Table 1). The overall pooled OR based on 2,529 advanced cases and 3,346 control subjects was 0.74 (95% CI=0.62–0.88). The reduction in risk associated with the II genotype was highly comparable among type 1 diabetic Caucasians (OR=0.72, 95% CI= 0.49–1.05), type 2 diabetic Caucasians (OR=0.68, 95% CI=0.39–1.16) and Asians with type 2 diabetes (OR= 0.76, 95% CI=0.59–

0.96), with the association in the latter subgroup being statistically significant (p=0.0229).

Discussion

The discovery of susceptibility genes for diabetic nephropathy could lead to novel avenues for the prevention and treatment of this debilitating complication [71]. Regarding ACE, an understanding of its genetic basis may facilitate the identification of specific patient subgroups that might benefit from targeted treatment with ACE inhibitors [4, 5]. Numerous investigations into the potential role of *ACE* as a susceptibility gene for diabetic nephropathy have been conducted over the past decade, with controversial results. Early meta-analyses attempted to reconcile these findings, but attempts to draw firm conclusions have been hindered by limited data, particularly when examining specific patient subgroups [9, 10].

Our present review of 47 studies reported since 1994 and comprising 14,727 subjects supports an overall association between the *ACE* Ins/Del polymorphism and diabetic nephropathy. However, the possible heterogeneity of this association between the Asian and Caucasian subgroups could suggest that a simple summary pooled OR across all studies may not be valid. Particularly, type 2 diabetic Asians who are II homozygotes had a 35% reduction in risk of diabetic nephropathy (defined on the minimal criterion of microalbuminuria) compared to those carrying the D allele. This positive finding is significantly different from that observed among Caucasians with type 2 diabetes (OR 0.90, 95% CI=0.78–1.04). Interestingly, when cases were defined on the basis of advanced diabetic nephropathy, the reduction in risk associated with the II genotype became more comparable across the three major subgroups with ORs ranging from 0.68 to 0.76; this association reached statistical significance among Asians with type 2 diabetes, who represented the largest dataset for this subgroup analysis. This consistent magnitude of risk reduction across subgroups could suggest that the use of a minimal-case definition according to incipient diabetic nephropathy might have inadvertently introduced greater heterogeneity among Caucasian cases than a definition rooted in advanced nephropathy. The widespread use of a minimal criterion of microalbuminuria as seen in our meta-analysis may have stemmed from earlier landmark studies, which showed that in the majority of diabetic patients, microalbuminuria inexorably led to overt proteinuria [72]. More recently, however, it has been reported that such patients may not necessarily progress to proteinuria but may instead stay microalbuminuric, or even regress to normoalbuminuria [73]. Furthermore, regression from micro- to normoalbuminuria could occur in a substantial proportion of patients, but regression from proteinuria appears much less frequently [74]. Aside from case heterogeneity, the possibility of the Ins/Del polymorphism being associated more strongly with early renal changes among Asians than in Caucasians may also be considered. Interestingly, all three previous meta-analyses reported in 1998 did not place specific emphasis on the use of advanced diabetic nephropathy as a basis for defining cases [9, 11]. This shortcoming may be attributed to the lack of the relevant studies at the time these reviews were performed [9, 10].

Several research groups have used control groups consisting solely of diabetic patients with normoalbuminuria despite a long duration of diabetes [38, 56]. This approach could yield clearer evidence for a true association between *ACE* Ins/Del and diabetic nephropathy, since the use of these controls can help reduce case misclassification. However, confining our analyses to studies with such 'long duration control groups' did not materially alter our findings. A possible explanation is that among studies that did not specify a requirement for a minimal diabetes duration, the control subjects may already have been diabetic for a significant period of time.

ACE inhibitors are already widely used in the clinical management of diabetic nephropathy. Several studies have examined how the effect of these drugs on diabetic nephropathy can be modulated by genetic variation at the *ACE* locus. Among these studies, Caucasians with the II genotype were reported to respond more favourably to ACE inhibitors in terms of a reduction in diabetes-associated albuminuria than carriers of the D allele [4]. This differential response to ACE inhibitor treatment was also observed in the EURODIAB Controlled Trial of Lisinopril in IDDM [5]. Curiously, while the D allele has been linked with higher ACE activity [6], several studies have indicated that the DD genotype could be associated with better response to the anti-proteinuric effect of ACE inhibitors in patients with diabetic nephropathy [75] and other renal diseases [76]. With these counter-intuitive findings, the precise manner in which the *ACE* genotype modifies response to ACE inhibitors remains unresolved. Clarification of this issue may conceivably be drawn from a more comprehensive haplotype analysis of the *ACE* locus that extends beyond the mere consideration of the Ins/Del polymorphism [77].

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