Is the Presence of Retinopathy of Practical Value in Defining Cases of Diabetic Nephropathy in Genetic Association Studies?

The Experience With the *ACE* Insertion/Deletion Polymorphism in 53 Studies Comprising 17,791 Subjects

Daniel P.K. Ng, Bee-Choo Tai, and Xiu-Li Lim

OBJECTIVE—A key consideration when setting up genetic studies is the case definition. For diabetic nephropathy, the case definition is typically based on the presence of albuminuria. However, it has been long debated whether diabetic nephropathy cases defined in this way may have a high prevalence of nondiabetic kidney disease, especially if diabetic retinopathy is absent.

RESEARCH DESIGN AND METHODS—We performed a meta-analysis of 53 studies comprising 17,791 subjects investigating the angiotensin-I converting enzyme insertion/deletion polymorphism, taking into account the requirement for diabetic retinopathy in the case definition and assuming a random-effects model.

RESULTS—No publication bias was observed. The overall pooled odds ratio (OR) for all 53 studies was 0.78 (95% CI 0.70–0.87; $P < 0.001$), which indicated a significant protection against diabetic nephropathy for genotype II compared with carriage of the D-allele. The pooled OR for the 11 studies ($n = 3,413$) requiring diabetic retinopathy in the case definition was 0.68 (0.53–0.86; $P = 0.002$), and this was not significantly different from the pooled OR of 0.81 (0.71–0.92; $P = 0.001$) obtained from the 42 remaining studies ($n = 14,378$) ($P = 0.198$). This lack of any significant effect of diabetic retinopathy was reiterated in subgroup analyses based on the type of diabetes present.

CONCLUSIONS—Stipulating the presence of diabetic retinopathy in the case definition of diabetic nephropathy did not appear to confer tangible benefits when detecting genetic associations. Besides reducing sample sizes, this stipulation makes the interpretation of genetic associations more difficult due to the potential confounding presence of diabetic retinopathy. ***Diabetes* 57: 2541–2546, 2008**

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Despite pharmacological interventions, diabetic nephropathy is the major cause of end-stage renal disease in many developed countries (1). The efficacy of such interventions may be dependent on patient genotypes, and epidemiological evidence firmly supports a role for genetic susceptibility in the development of diabetic nephropathy in both type 1 and type 2 diabetes (2). Identification of the genes responsible holds the promise for greater insight into the pathophysiology of this debilitating complication and may ultimately provide novel therapies for disease prevention and intervention.

A key consideration when setting up genetic studies for diabetic nephropathy is the case definition. Because diabetic nephropathy is rarely diagnosed using invasive kidney biopsies, the case definition of this complication in genetic studies is typically based on the presence of albuminuria (3). However, applying this case definition, it is plausible that there is a substantial number of subjects who were classified as having diabetic nephropathy but actually have nondiabetic kidney disease instead. This misclassification in genetic studies will be expected to drive any true association toward the null. In an attempt to circumvent this problem, certain investigators have proposed that diabetic nephropathy cases should be required to have diabetic retinopathy as well. The rationale for this proposal is that several studies have suggested that albuminuria can be attributed with confidence to diabetic nephropathy if diabetic retinopathy is present (4).

The vital question remains whether the stipulation of diabetic retinopathy does indeed facilitate the identification of susceptibility genes for diabetic nephropathy in real-life association studies. To address this issue, we performed a meta-analysis on the association between diabetic nephropathy and the *ACE* insertion/deletion polymorphism (*ACE* I/D), taking diabetic retinopathy status into account. This genetic marker is the most extensively studied polymorphism to date for diabetic nephropathy; as such, data from 53 studies comprising 17,791 subjects were available for this meta-analysis.

RESEARCH DESIGN AND METHODS

We used a preexisting dataset based on 47 studies published from January 1994 through March 2004 that examined the association between *ACE* I/D and diabetic nephropathy (3). This dataset was subsequently expanded in 2006 by the addition of six later studies to a total of 53 studies comprising 17,791

TABLE 1
Summary of 53 studies on *ACE* I/D and diabetic neuropathy

Lead author	Year	Diabetes type	Case definition requires DR	Case subjects with DR (%)	Control definition requires DR	Control subjects with DR (%)	Case genotypes (*n*)			Control genotypes (*n*)		
							DD	ID	II	DD	ID	II
Doria	1994	Type 1	No	70.0	No	21.0	24	35	15	16	41	20
Powrie	1994	Type 1	No	NA	No	NA	7	8	4	24	37	24
Dudley	1995	Type 2	No	22.1	No	19.0	47	85	31	70	148	49
Fujisawa	1995	Type 2	No	NA	No	NA	7	23	24	6	12	17
Mizuiri	1995	Type 2	Yes	100.0	No	NA	19	50	11	9	11	11
Panagiotopoulos	1995	Type 2	No	NA	No	NA	15	25	10	42	44	29
Schmidt	1995	Type 1	No	74.6	No	63.9	52	38	24	55	55	23
Tarnow	1995	Type 1	Yes	100.0	No	65.0	63	95	40	67	77	46
Rabensteiner	1995	Type 1	No	NA	No	NA	16	39	9	8	33	15
Chowdhury	1996	Type 1	Yes	100.0	No	NA	78	124	40	55	79	32
Doi	1996	Type 2	No	93.9	No	69.4	29	85	50	12	56	56
Nakajima	1996	Type 2	No	NA	No	NA	14	50	37	4	19	18
Oh	1996	Type 1	No	83.9	No	42.9	10	9	12	7	10	11
Ohno	1996	Type 2	No	58.2	No	37.7	15	38	26	5	15	33
Yoshida	1996	Type 2	Yes	100.0	No	48.0	19	28	25	7	46	43
Barnas	1997	Type 1	No	100.0	No	78.0	14	27	9	4	21	15
Hibberd	1997	Type 1	Yes	100.0	No	46.5	21	42	9	36	43	7
Jeffers	1997	Type 2	No	NA	No	NA	23	20	7	139	218	102
Marre	1997	Type 1	Yes	100.0	Yes	100.0	119	168	50	48	69	40
Ringel	1997	Type 1	No	41.0	No	20.4	35	68	31	57	130	39
Ringel	1997	Type 2	No	35.4	No	15.0	44	84	33	35	69	36
Demurov	1997	Type 1	No	NA	No	NA	24	29	3	24	32	20
Schmidt	1997	Type 2	No	64.7	No	35.3	121	129	61	131	154	62
Pfohl	1998	Type 1	No	87.0	No	87.0	17	15	8	15	18	7
Freire	1998	Type 1	No	38.0	No	10.0	33	32	12	34	45	10
Grzeszczak	1998	Type 2	No	48.9	No	39.2	129	230	103	73	118	63
Hanyu	1998	Type 2	Yes	100.0	Yes	100.0	4	13	7	2	5	14
Huang	1998	Type 2	No	NA	No	NA	11	16	2	20	25	9
Wu	1998	Type 2	No	NA	No	NA	12	18	21	1	11	6
Bouhanick	1999	Type 1	No	NA	No	NA	4	5	4	19	34	10
De Cosmo	1999	Type 1	Yes	100.0	No	NA	73	79	23	65	53	18
Kuramoto	1999	Type 2	No	42.4	No	13.8	9	16	8	3	13	13
Miura	1999	Type 1	No	71.4	No	44.7	13	49	36	10	58	35
Vleming	1999	Type 1	No	100.0	No	NA	39	24	16	26	34	22
Wong	1999	Type 2	No	96.0	No	30.0	7	30	43	12	40	36
Hsieh	2000	Type 2	No	NA	No	NA	40	59	80	21	50	86
van Ittersum	2000	Type 1	No	71.0	No	28.2	13	33	23	49	86	53
Araz	2001	Type 2	No	70.0	No	31.7	34	64	18	43	57	23
Azar	2001	Type 1	No	NA	No	NA	23	27	2	1	7	2
Gohda	2001	Type 2	No	NA	No	NA	85	222	229	31	92	89
Taniwaki	2001	Type 2	No	84.9	No	72.5	14	40	32	12	26	31
Viswanathan	2001	Type 2	Yes	100.0	No*	0.0	24	45	17	5	8	10
Fradin	2002	Type 2	No	35.0	No	19.5	38	61	18	44	54	20
Lee	2002	Type 2	No	NA	No	NA	40	137	117	39	170	208
Ha	2003	Type 2	Yes	100.0	No	39.4	43	62	35	9	57	33
Hadjadj	2003	Type 2	No	4.5	No	2.0	1119	1468	552	208	282	115
Okuno	2003	Type 2	No	50.0	No	26.3	3	8	1	5	12	21
Arzu Ergen	2004	Type 2	No	16.0	No	22.0	9	11	5	24	21	5
Degirmenci	2005	Type 2	No	NA	No	NA	12	25	6	30	47	19
Shestakova	2005	Type 1	No	NA	No	NA	13	35	15	12	30	24
Canani	2005	Type 2	Yes	100.0	No	NA	126	181	66	181	308	120
Wang	2005	Type 2	No	77.9	No	NA	19	43	36	128	496	559
Ng	2006	Type 2	No	NA	No	NA	96	148	47	52	83	32

The first 47 studies have been previously referenced (ref. 3). *Absence of diabetic retinopathy specifically required in control subjects of this study. DR, diabetic retinopathy; NA, information on diabetic retinopathy not available.

subjects (5–10). Briefly, studies were considered if they provided sufficient information for a comparison of the *ACE* I/D genotype distribution between case and control subjects. Case subjects were type 1 or type 2 diabetic subjects fulfilling the minimal criterion of microalbuminuria, whereas control subjects were defined predominantly on the basis of normoalbuminuria. Of the 53 studies, 11 specifically required the concomitant presence of diabetic retinopathy when defining cases of diabetic nephropathy (Table 1).

Statistical analyses. Funnel plots of the effect estimate based on log-odds ratio were plotted against its SE to evaluate the possibility of publication bias (11). The magnitude of the genetic association between *ACE* I/D and diabetic nephropathy was obtained by calculating the odds ratio (OR) and its associated 95% CI. A random effects model was used based on the assumption that the studies represented a random sample from the larger population of such studies, with each having its own underlying effect size. Under this model, it

is assumed that the study-specific OR varies in response to a mean population effect size. Because the random effects model takes into account the inter-study heterogeneity, such as differences in study design and case definitions for diabetic nephropathy, it provides a more conservative evaluation of the significance of the association than one based on fixed effects (12).

RESULTS

A total of 53 studies (n = 9,556 case and 8,235 control subjects) fulfilled the criteria for inclusion in this review (Table 1). Twenty-one studies involved type 1 diabetic subjects (n = 4,154), while the remaining 32 studies were conducted on patients with type 2 diabetes (n = 13,637). The potential presence of publication bias was assessed using funnel plots of the estimate of log-odds ratio for the genotype II versus DD/ID against its SE (Fig. 1*A*). Considerable scatter was observed around the pooled log-odds ratio estimate when the reciprocal of the SE was small and approached convergence to form a symmetrical funnel, as this reciprocal increased when all 53 studies were assessed. Similarly, there was no evidence of such bias when the 53 studies were analyzed separately, depending on whether they required the concomitant diabetic retinopathy in the case definitions (Fig. 1*B* and 1*C*).

The overall pooled OR for all 53 studies was 0.78 (95% CI 0.70–0.87; $P < 0.001$), which indicated a significant protection against diabetic nephropathy for genotype II compared with carriage of the D-allele (Fig. 2). The pooled OR for the 11 studies (n = 3,413) requiring diabetic retinopathy in the case definition was 0.68 (0.53–0.86; P = 0.002), and this was not significantly different from the pooled OR of 0.81 (0.71–0.92; P = 0.001) obtained from the 42 remaining studies (n = 14,378) that eschewed the corroborative presence of diabetic retinopathy (P = 0.198) (Fig. 2).

In subgroup analyses on 21 studies conducted on 4,154 type 1 diabetic patients, the overall pooled OR was 0.84 (95% CI 0.68–1.05; P = 0.119). The pooled OR for five studies (n = 1,759) requiring diabetic retinopathy status in case subjects was 0.78 (0.58–1.06; P = 0.110), and this was similar to the pooled OR of 0.85 (0.63–1.13; P = 0.255) for the remaining 16 type 1 diabetes studies (n = 2,395) (P = 0.704). In corresponding subgroup analyses, the overall pooled OR was 0.75 (0.66–0.86; $P < 0.001$) for the 32 studies comprising 13,637 type 2 diabetic patients. The pooled OR for six studies (n = 1,654) requiring diabetic retinopathy in the case definitions was 0.54 (0.36–0.82; P = 0.004), which was not significantly smaller than the pooled OR of 0.79 (0.69–0.91; P = 0.001) for the 26 remaining type 2 diabetes studies (n = 11,983) (P = 0.087).

We considered the possibility that this lack of effect of diabetic retinopathy may be due to the fact that case subjects in some studies may have had a high prevalence of this complication even though it was not explicitly required in the case definition. Because these studies would have been placed under the category of studies not requiring diabetic retinopathy in the preceding analyses, the anticipated outcome would have been to drive any apparent effect of diabetic retinopathy toward the null. To clarify this issue, we scrutinized the published reports and found that of the 42 studies that did not specifically stipulate diabetic retinopathy in the case definition, 25 did provide sufficient clinical information for us to determine the prevalence of diabetic retinopathy among the case subjects (Table 1). Seven studies, in which diabetic retinopathy was present in at least a majority (80%) of cases,

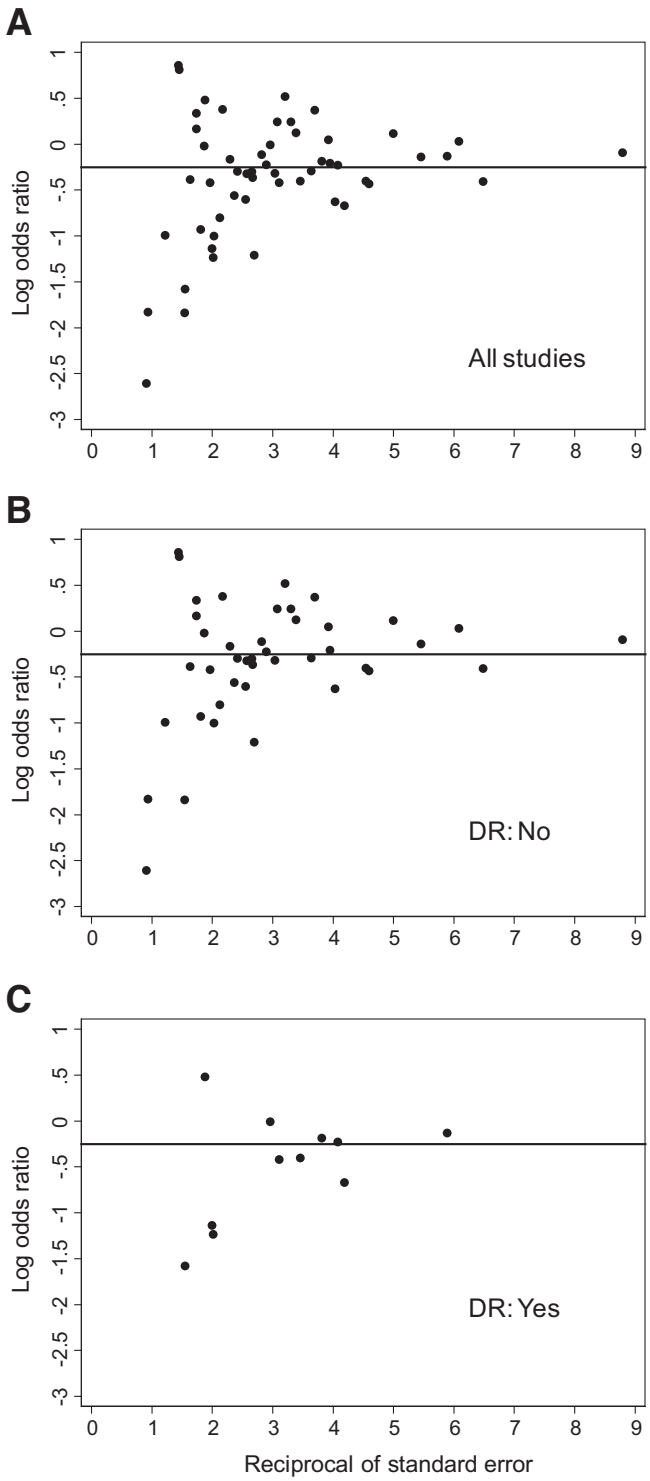

FIG. 1. Funnel plot for the evaluation of publication bias in studies of association of *ACE* I/D for all 53 studies (*A*), 42 studies not requiring retinopathy in the case definition (*B*), and 11 studies requiring diabetic retinopathy to corroborate the presence of diabetic nephropathy (*C*). DR, diabetic retinopathy.

were selected from among these (Table 1). These studies were combined with the 11 studies that specified diabetic retinopathy in their case definitions for comparison with the other remaining studies. The overall pooled ORs for these 18 studies (n = 4,414) was 0.71 (95% CI 0.58–0.87; P = 0.001) compared with 0.82 (0.72–0.94; P = 0.003) for the 35 remaining studies (n = 13,377) (P = 0.249) (online appendix supplementary Fig. 1 available at http://dx.doi.

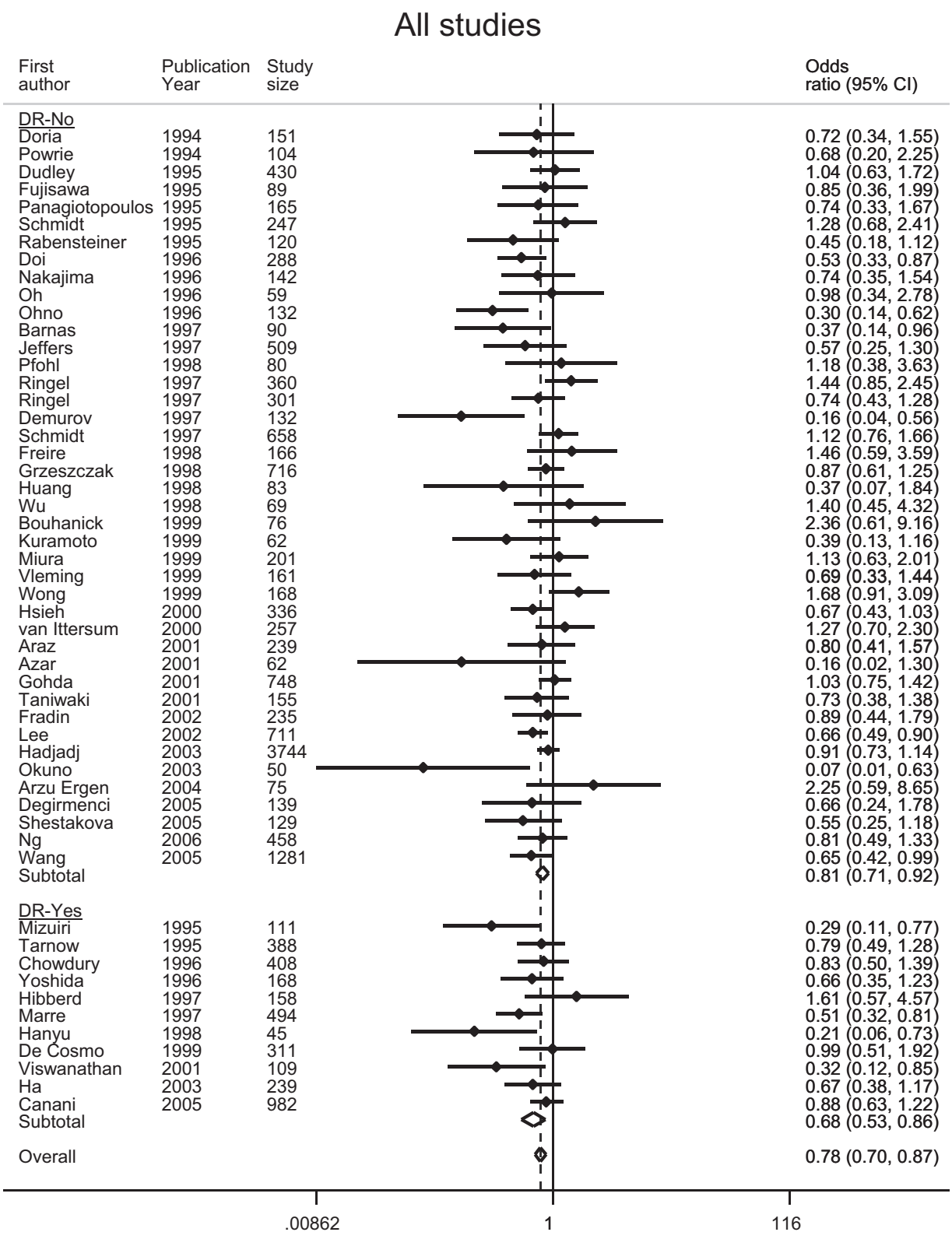

FIG. 2. OR and the associated 95% CI comparing *ACE* II with ID/DD genotypes in all 53 studies, which comprised 42 studies not requiring diabetic retinopathy in the case definition and 11 studies requiring diabetic retinopathy (DR).

org/10.2337/db08-0581]). Confining our analyses to just the 36 studies that provided information about diabetic retinopathy also yielded similar findings (data not shown). No significant differences associated with the requirement for diabetic retinopathy were observed in either patients with type 1 ($P = 0.448$) or type 2 diabetes ($P = 0.236$).

DISCUSSION

The promise of new insights into the pathogenesis of diabetic nephropathy is fuelling intense efforts to identify genes conferring risk of the complication (13–15). While much of the attention has been placed on attaining large sample sizes to provide power for detecting small effects,

another key consideration is the case definition of diabetic nephropathy. In this study, we reviewed the literature on the association of *ACE* I/D and diabetic nephropathy and found evidence suggesting that stipulating the concomitant presence of diabetic retinopathy in order to corroborate a diagnosis of diabetic nephropathy is unlikely to yield significant benefits when searching for genetic associations.

The inclusion of diabetic retinopathy in the case definition is commonplace in the published literature on *ACE* I/D. Of the 53 studies, 21% imposed this requirement, and this was comparable in studies focusing on either type 1 or type 2 diabetic patients (24 and 19% respectively). This practice is likely based on several studies that found that only a subset of patients with proteinuria and/or azotemia have kidney biopsies that substantiated a diagnosis of diabetic glomerulopathy, which has subsequently been taken to mean that proteinuria per se is insufficient as conclusive evidence of diabetic nephropathy (16–20). However, in a systematic review of nine published reports and their data (21), Oslen and Mogensen deliberated on this issue and proposed that a very likely reason for the high prevalence of nondiabetic kidney disease was the fact that most of the reports were based on biased groups of patients who were inadvertently selected for such nondiabetic kidney conditions (21). Another potential explanatory factor was the application of a differing criterion for diagnosing glomerulonephritis, a major contributor to nondiabetic kidney disease (21).

In our literature review, several points emerged that should be highlighted. Of the 11 studies that required diabetic retinopathy in the case definition, 9 studies did not require that their control subjects have diabetic retinopathy as well. It was also striking that one study specifically required that its control subjects be free of diabetic retinopathy when all its case subjects had this eye complication (Table 1). Understandably, one would be hard pressed to determine whether any observed association between *ACE* I/D and diabetic nephropathy, diabetic retinopathy, or even a combination of both complications truly exists.

In practical terms, the requirement for diabetic retinopathy in control subjects will inadvertently diminish the overall size of the study population, which is already limited by the requirement that case subjects have diabetic retinopathy. Unfortunately, on the basis of our present results, this drop in sample size and consequent drop in power come without any tangible reciprocal benefit that would be expected if disease misclassification among cases had been rampant in the absence of diabetic retinopathy as previously suggested (20). Moreover, because recent studies suggested that the majority (70–74%) of albuminuric type 2 diabetic patients do indeed have diabetic glomerulopathy even in the absence of diabetic retinopathy (22,23), it becomes questionable whether genetic associations found in studies using diabetic retinopathy can be readily extrapolated to these diabetic nephropathy patients. Nevertheless, it is noteworthy that the overall pooled OR was slightly but consistently higher in studies where diabetic retinopathy was prevalent, although even with the large dataset under review, this difference failed to reach statistical significance. One may thus consider the possibility that including diabetic retinopathy helps in the identification of potential genetic factors for common underlying traits that may manifest as a joint retinal-renal phenotype.

Several strengths and limitations of our study should be discussed. On a positive note, the meta-analysis was conducted on a substantial dataset comprising 17,791 patients from 53 studies. Moreover, there was no overt sign of publication bias that would argue against the validity of our results, with funnel plot analyses indicating that small studies with negative findings were as likely to be published as large studies with positive findings. In addition, we performed subgroup analyses according to whether the patients had type 1 or type 2 diabetes. This distinction was relevant because of the debate as to whether nondiabetic kidney disease is more common in albuminuric patients with type 1 or type 2 diabetes (16,24).

A main limitation is that our study was restricted to *ACE* I/D. This decision was borne of necessity because *ACE* I/D is the most extensively studied polymorphism to date with regards to diabetic nephropathy, and there is a severe lack of extensive studies into other genetic markers. Despite this situation, our study manages to render a first critical insight into the issue. Finally, reports of late have provided evidence that diabetic nephropathy may be associated with specific risk haplotypes at the *ACE* locus. However, a meta-analysis on *ACE* haplotypes is precluded due to a paucity of such reports (10,25).

In conclusion, our study using real-life association data suggests that the presence of diabetic retinopathy may be of limited practical value for defining cases of diabetic nephropathy when seeking genetic associations. In addition, the reduced sample sizes arising from such a stipulation may make it harder to detect these associations. Interpretation of the results from such studies could also be hampered by the possible confounding presence of diabetic retinopathy if left uncontrolled.

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