Diabetic nephropathy : pathology, genetics and carnosine metabolism

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Genetic associations in diabetic nephropathy: a meta-analysis

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Abstract

Aims/hypothesis This meta-analysis assessed the pooled effect of each genetic variant reproducibly associated with diabetic nephropathy.

Methods PubMed, EMBASE and Web of Science were searched for articles assessing the association between genes and diabetic nephropathy. All genetic variants statistically associated with diabetic nephropathy in an initial study, then independently reproduced in at least one additional study, were selected. Subsequently, all studies assessing these variants were included. The association between these variants and diabetic nephropathy (defined as macroalbuminuria/proteinuria or end-stage renal disease [ESRD]) was calculated at the allele level and the main measure of effect was a pooled odds ratio. Pre-specified subgroup analyses were performed, stratifying for type 1/type 2 diabetes mellitus, proteinuria/ESRD and ethnic group.

Results The literature search yielded 3455 citations, of which 671 were genetic association studies investigating diabetic nephropathy. We identified 34 replicated genetic variants. Of these, 21 remained significantly associated with diabetic nephropathy in a random-effects meta-analysis. These variants were in or near the following genes: *ACE, AKR1B1* (two variants)*, APOC1, APOE, EPO, NOS3* (two variants)*, HSPG2, VEGFA, FRMD3* (two variants)*, CARS* (two variants)*, UNC13B, 'CPVL and CHN2'* and *GREM1*, plus four variants not near genes. The odds ratios of associated genetic variants ranged from 0.48 to 1.70. Additional variants were detected in subgroup analyses: *ELMO1* (Asians)*, CCR5* (Asians) and *CNDP1* (type 2 diabetes).

Conclusions/interpretation This meta-analysis found 24 genetic variants associated with diabetic nephropathy. The relative contribution and relevance of the identified genes in the pathogenesis of diabetic nephropathy should be the focus of future studies.

Introduction

Diabetes mellitus has rapidly increased to epidemic proportions over the past few decades. The number of patients with diabetes mellitus worldwide was estimated at 173 million in 2002 and is predicted to increase to 350 million cases by 2030 (1). Diabetes mellitus is associated with severe complications including nephropathy, neuropathy, retinopathy and accelerated cardiovascular disease.

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in developed countries (1). Although glycaemic control inversely relates to the degree of microvascular complications including diabetic nephropathy (2), some patients appear to be at increased risk. The majority of patients with type 1 diabetes mellitus will either develop diabetic nephropathy within the first 15 years after diagnosis or will remain relatively protected thereafter (3). Differential disease risk in diabetic nephropathy may be partly attributable to genetic susceptibility. Evidence for a genetic component to diabetic nephropathy comes from family studies displaying familial aggregation of diabetic nephropathy both in type 1 and in type 2 diabetes mellitus (4-6), as well as differences in the prevalence of diabetic nephropathy between ethnic groups (7,8).

The literature involving genetic associations in complex disease has been plagued by inconsistencies (9). Small sample sizes and false positive results were often responsible for lack of reproducibility (10). In addition, the prior probabilities of genetic associations are low. Therefore, the number of false positive associations generated by chance alone is high, particularly when low prior probabilities were not accounted for in the statistical analyses. (11). Incorrect phenotyping may also lead to spurious results. Thus independent replication of association remains essential in order to avoid false positive associations. The aim of this meta-analysis was to assess the pooled effect of genetic variants that have reproducibly been associated with diabetic nephropathy.

Methods

Eligibility criteria

We searched for studies comparing genetic variants in diabetes mellitus patients with diabetic nephropathy, relative to diabetes mellitus patients without diabetic nephropathy. We limited our analyses to studies investigating established and advanced diabetic

nephropathy. To be included, all cases in the report had to have diabetes mellitus with macroalbuminuria and/or overt proteinuria, ESRD attributed to diabetic nephropathy or biopsy-proven diabetic nephropathy. In addition, diabetic control participants had to have either: (1) normoalbuminuria; (2) normoalbuminuria or microalbuminuria after >15 years diabetes mellitus duration (microalbuminuria developing after >15 years diabetes mellitus duration is a poor predictor of diabetic nephropathy (3)); (3) stable kidney function (serum creatinine < 106.1 μmol/L) after >15 years of diabetes mellitus, irrespective of albuminuria.

Studies were excluded when the control group consisted of non-diabetic participants, since in that case genetic associations ascribed to diabetic nephropathy could have been due to diabetes mellitus. Follow-up and case–control studies were both eligible for inclusion in the meta-analysis.

Literature search and data collection

A search strategy was devised in collaboration with a trained librarian. The following databases were searched: PubMed (1949 to April 2010), EMBASE (OVID-version, 1980 to April 2010) and Web of Science (1945 to April 2010). The search strategy consisted of multiple queries combining: 'Diabetic Nephropathy' and 'Genes' or 'Polymorphisms'. For these two concepts, all relevant keyword variations were used. In addition, the names of specific genes and polymorphisms were combined with the topic 'Diabetic Nephropathy'. This search strategy was optimised for every database consulted, taking into account differences in the various controlled vocabularies and different database-specific technical variations. The search was performed in April 2010. To ensure maximum sensitivity, limits or filters were not placed on the searches. Language restrictions were not included in the initial search. References of other narrative and systematic reviews were also checked for relevant articles. The search strategy was updated if a reference was missing. The process was performed three times to ensure that no references were omitted.

Two authors (A. L. Mooyaart and E. J. J. Valk) of this study independently reviewed the titles and abstracts of the citations to identify genetic association studies. Genetic association studies were screened for whether the study contained a positive or a negative association between the genetic variant and diabetic nephropathy (association defined as significant at $p<0.05$). When a genetic variant was found to be associated with established or advanced diabetic nephropathy (either at the allelic or genotypic

level, including the recessive and dominant model) in two independent studies, that variant was considered to be a reproduced genetic variant. For these replicated variants, all other genetic studies were identified to estimate the effect of the variant on diabetic nephropathy, irrespective of *p* values.

Data extraction and analysis

The main outcome of the meta-analysis was the pooled odds ratio for the association between reproduced genetic variants and diabetic nephropathy. Odds ratios were calculated at allele level and not at genotype level. Of the reproduced genes, allele frequencies were extracted from studies. For single nucleotide polymorphisms (SNPs), the frequency of the minor allele was compared between diabetic nephropathy cases and non-nephropathy diabetic controls. For other genetic variants such as microsatellites, we compared the allele between cases and controls, as used in the literature and other meta-analyses (12, 13). The random-effects model was performed by default. Heterogeneity within the studies was estimated by the I^2, which is the percentage of the total variation across studies that is due to heterogeneity rather than chance. An I^2 of 25%, 50% and 75% was considered low, moderate and high respectively (14). Pre-specified stratified analyses were performed to explain heterogeneity or investigate whether the reported association was present in a subgroup. Stratified analysis was performed for diabetes mellitus type (type 1 or type 2), diabetic nephropathy stage (macroalbuminuria and/or overt proteinuria, established diabetic nephropathy and ESRD [advanced diabetic nephropathy]) (15) and ethnicity (European vs Asian origin). The subgroup analysis was only included in this study if the association between the genetic variant and diabetic nephropathy was reproduced in that subgroup. We tested for publication bias using the Begg and Egger test and provided funnel plots of all genetic variants which were reproducibly associated with diabetic nephropathy. It should, however, be noted that funnel plot asymmetry can have other causes than publication bias (16). Furthermore, the effect of ethnicity was assessed if there were sufficient data by metaregression. Most analyses were performed in Review Manager (RevMan) Version 5.0 (The Nordic Cochrane Centre, Copenhagen, Denmark; The Cochrane Collaboration, 2008), except for the analysis of publication bias and metaregression, which was performed in STATA 10.0 (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataC).

Results

Initial search and results

The initial literature search yielded 3455 citations, 671 of which were genetic association studies investigating diabetic nephropathy in humans (Fig. 1). In these studies, we identified 34 reproduced genetic variants in 24 genes associated with diabetic nephropathy. Data on at least one of these 34 variants were found in 132 articles, representing 153 studies. Only three follow-up studies were included. All other studies were case–control studies. The maximum number of studies in an article was five. References of all articles and details of these studies are shown in the Electronic supplementary material (ESM) Tables 1 and 2. The 132 articles were published between 1994 and 2010. The number of cases included in these articles ranged from four to 1572, and in a study from four to 656 cases. Of the 34 reproduced genetic variants, 21 genetic variants in or near 16 genes were significantly associated with diabetic nephropathy after random-effects meta-analysis (Fig. 2a). An overview of the pooled odds ratios of all reproduced variants in relation to diabetic nephropathy is shown in Fig. 2a, b. The odds ratios of the significant associations with diabetic nephropathy ranged between 0.48 and 0.78 for protective effects, and 1.12 to 1.70 for risk effects. Figure 3 contains an overview of the pooled odds ratios of all reproduced variants in relation to diabetic nephropathy among subgroups. Three reproduced variants were not significantly associated with diabetic nephropathy in the whole population after meta-analyses, but were associated in one subgroup: rs1799987 of *CCR5* and rs741301 of *ELMO1* in the Asian subgroup, and D18S880 of the *CNDP1* gene in patients with type 2 diabetes mellitus. Details of analyses of all assessed genetic variants are provided in Table 1. Forest plots of all individual genetic variants and funnel plots for publication bias, as well as results of meta-regression for ethnicity are shown in the ESM (ESM Figs 1–36). If the meta-analysis revealed a positive association between a given genetic variant and diabetic nephropathy, and more than ten studies investigating that variant in relation to diabetic nephropathy were available, a metaregression was performed. Only three genetic variants fitted the above-mentioned criteria (*ACE* rs179975, *AKRB1* CA repeat Z–2, *APOE* E2/3/4). In these variants, metaregression showed that ethnicity did not explain the heterogeneity (ESM Figs 1–36).

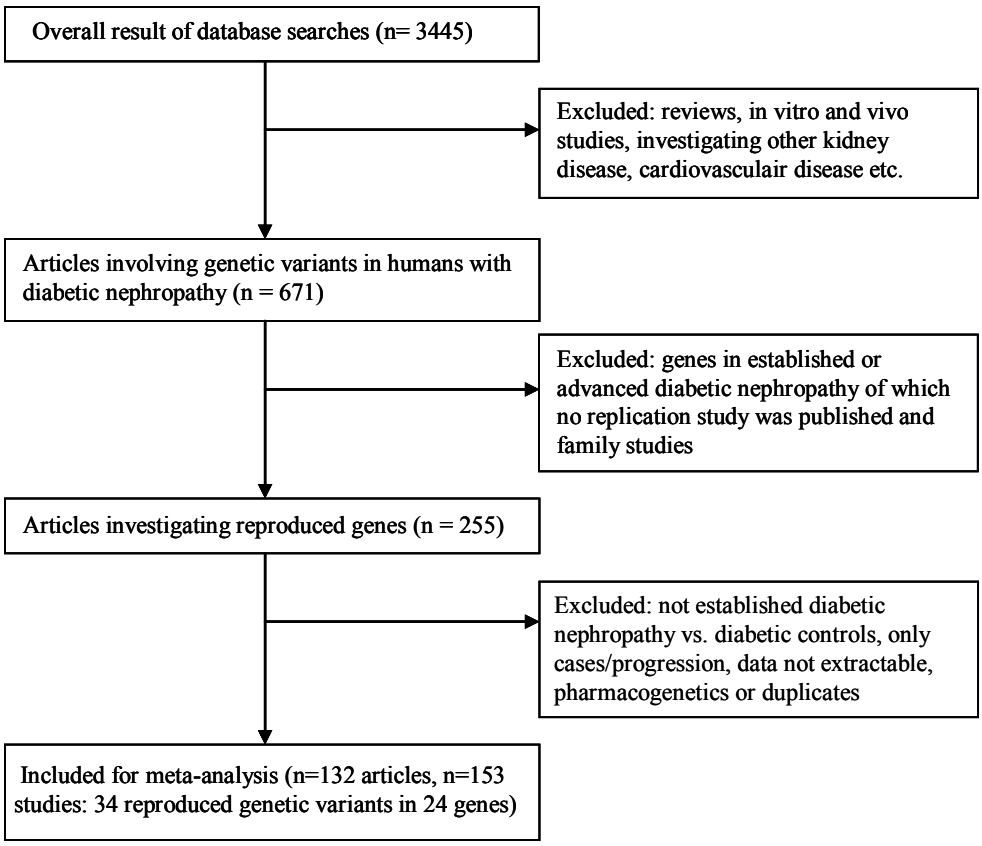

Figure 1. Flowchart showing how studies were selected for meta-analysis

Table 1. Details of reproduced variants after meta-analysis, including subgroups

Variants per gene	Minor allele	Total/subgroup	Studies (*n*)	Cases (*n*)	Controls (*n*)	OR (95%CI)	I^2 (%)	*p* value[a]
ACE								
rs179975	Deletion	Total	42	5721	7798	1.24 (1.12-1.37)	66	0.061
		Type 1 diabetes	14	2215	2685	1.13 (1.04-1.23)	44	-
		Type 2 diabetes	28	3506	5113	1.33 (1.16-1.52)	71	-
		ESRD	10	1405	1367	1.39 (1.21-1.6)	29	-
		Proteinuria	30	4071	5593	1.20 (1.07-1.36)	67	-
		Europeans	17	2660	3221	1.10 (0.99-1.22)	38	-
		Asians	19	2465	3397	1.28 (1.10-1.56)	65	-
ACACB								
rs2268388	T	Total	5	1007	900	0.83 (0.62-1.11)	77	0.624
ADIPOQ								
rs17300539	A	Total	3	1104	1138	1.37 (0.93-2.03)	67	0.117
AGT								
rs699	C	Total	21	4117	4800	1.10 (0.96-1.25)	71	0.85
		Type 2 diabetes	10	1966	2309	1.11 (0.85-1.45)	83	-
		Asian	8	1717	1933	1.10 (0.78-1.55)	87	-
AGTR1								
rs5186	C	Total	15	3220	3501	0.99 (0.91-1.08)	40	0.102
		European	10	1564	2038	1.06 (0.94-1.19)	36	-
		Type 1 diabetes	9	1525	1920	1.04 (0.92-1.17)	30	-
AKR1B1								
CA repeat	Z–2	Total	19	2237	3017	1.12 (1.02-1.24)	11	0.807
		Type 1 diabetes	10	1380	1308	1.12 (1.00-1.25)	16	-
		European	14	1654	1854	1.08 (0.95-1.21)	18	-
	Z+2	Total	17	1894	2005	0.83 (0.66-1.03)	59	0.805
		Type 1 diabetes	10	1380	1308	0.79 (0.68-0.92)	60	-
		European	11	1513	1557	0.81 (0.66-0.99)	40	-
rs759853	T	Total	9	1243	1933	1.40 (1.13-1.74)	67	0.144
		Type 1 diabetes	4	636	537	1.58 (1.01-2.46)	84	-
		European	6	854	913	1.45 (1.07-1.97)	75	-

APOC1								
rs4420638	G	Total	2	857	935	1.54 (1.29-1.83)	0	0.317
APOE								
E2, E3, E4	E2	Total	11	1257	1555	1.70 (1.12-2.58)	68	0.186
		Type 2 diabetes	5	368	751	2.21 (1.22-4.00)	42	-
		Asians	4	312	722	2.35 (1.29-4.30)	48	-
		Europeantype 1 diabetes	6	889	803	1.48 (0.84-2.58)	77	-
	E4	Total	11	1257	1555	0.78 (0.62-0.98)	40	0.186
CARS								
rs451041	A	Total	3	1052	2057	1.37 (1.21-1.54)	0	0.117
rs739401	C	Total	2	820	885	1.32 (1.15-1.51)	0	0.317
CCR5								
rs1799987	G	Total	9	2562	2965	0.81 (0.66-1.00)	85	0.012
		Asians	4	627	907	0.58 (0.43-0.76)	69	-
CNDP1								
D18S880	5L	Total	7	2603	3136	0.92 (0.82-1.01)	34	0.176
		Type 2 diabetes	2	344	329	0.77 (0.61-0.97)	0	-
'CPVL and CHN2'								
rs39059	G	Total	2	820	885	0.74 (0.64-0.85)	0	0.317
rs39075	A	Total	3	1052	2057	0.84 (0.62-1.14)	84	0.602
ELMO1								
rs741301	G	Total	3	1366	1219	1.31 (0.82-2.08)	91	0.602
		Asians	2	546	334	1.58 (1.28-1.94)	0	-
EPO								
rs1617640	T	Total	3	1618	954	0.67 (0.60-0.76)	0	0.117
		Type 1 diabetes	2	1244	715	0.67 (0.58-0.76)	0	-
FRMD3								
rs1888747	C	Total	3	1052	2057	0.74 (0.65-0.83)	0	0.602
rs10868025	A	Total	3	1052	2057	0.72 (0.64-0.81)	11	0.117
GLUT1								
rs841853	T	Total	7	867	1035	1.10 (0.89-1.35)	53	0.881
GREM1								
rs1129456	T	Total	2	859	940	1.53 (1.25-1.89)	0	0.317

Table 1. Continued

Variants per gene	Minor allele	Total/subgroup	Studies (*n*)	Cases (*n*)	Controls (*n*)	OR (95%CI)	I^2 (%)	*p* value[a]
HP								
Hp 1/2	Hp 1	Total	8	718	1056	0.81 (0.60-1.08)	62	0.322
HSPG2								
rs3767140	G	Total	4	732	381	0.72 (0.59-0.87)	0	0.384
		European type 1 diabetes	2	417	240	0.64 (0.49-0.84)	0	-
NOS3								
rs2070744	C	Total	2	273	450	1.39 (1.09-1.78)	0	0.216
rs3138808	a-deletion 393 bp	Total	8	1250	1368	1.31 (1.02-1.67)	53	0.317
		European type 1 diabetes	3	679	657	1.45 (0.97-2.17)	69	-
PPARG								
rs1801282	G	Total	7	2468	2394	0.84 (0.65-1.10)	78	0.024
PVT1								
rs11993333	T	Total	2	628	661	0.65 (0.39-1.10)	80	0.317
UNC13B								
rs13293564	T	Total	4	1572	1910	1.23 (1.11-1.35)	0	1.00
VEGFA								
rs833061	C	Total	2	242	301	0.48 (0.37-0.61)	0	0.317
No gene								
rs1041466	G	Total	3	1052	2057	1.38 (1.21-1.58)	0	0.602
rs1411766	A	Total	3	1052	2057	1.36 (1.20-1.54)	0	0.117
rs7989848	A	Total	3	1052	2057	1.32 (1.16-1.51)	0	0.117
rs9521445	A	Total	2	820	885	1.35 (1.18-1.55)	0	0.317
rs6492208	C	Total	3	1052	2057	0.85 (0.67-1.06)	0	0.117

[a]Begg test for funnel plot asymmetry, which is suggestive of publication bias

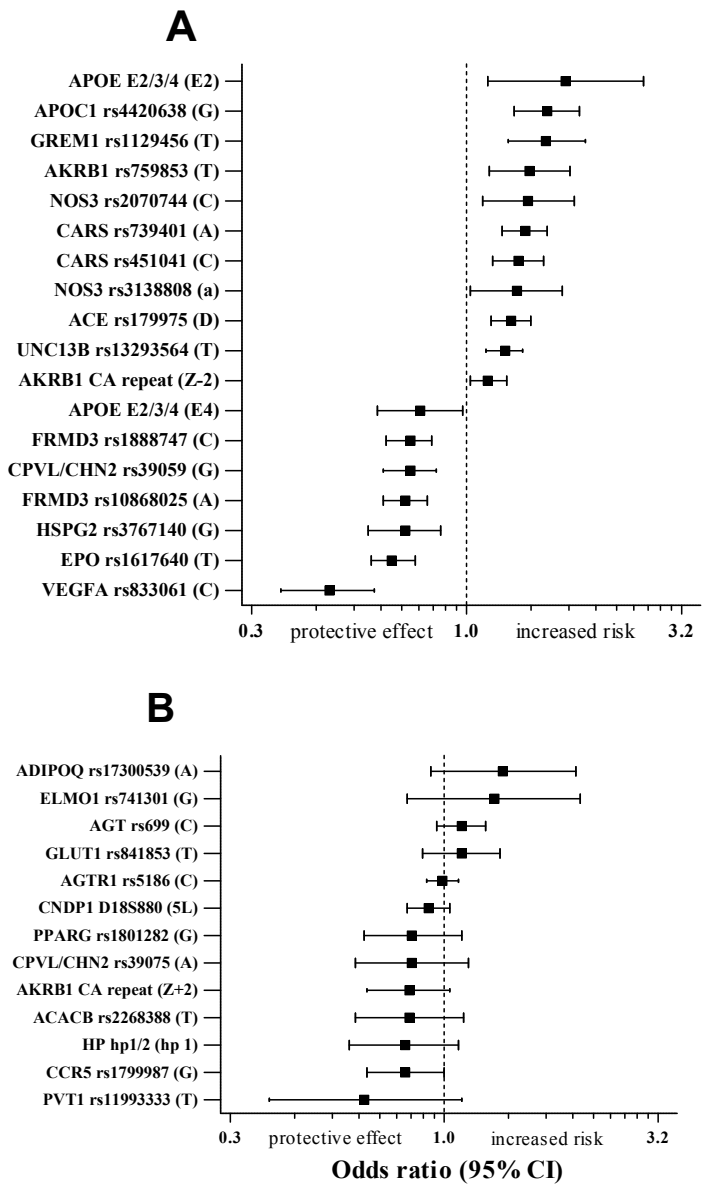

Figure 2. Genetic variants reproducibly associated with diabetic nephropathy. **a** All genetic variants in or near a gene that were reproduced in an independent study and significantly associated with diabetic nephropathy after meta-analysis. **b** All genetic variants in or near a gene that were reproduced in an independent study, but were not significantly associated with diabetic nephropathy after meta-analysis. Parentheses (*y*-axis labelling) contain the allele used in the comparison

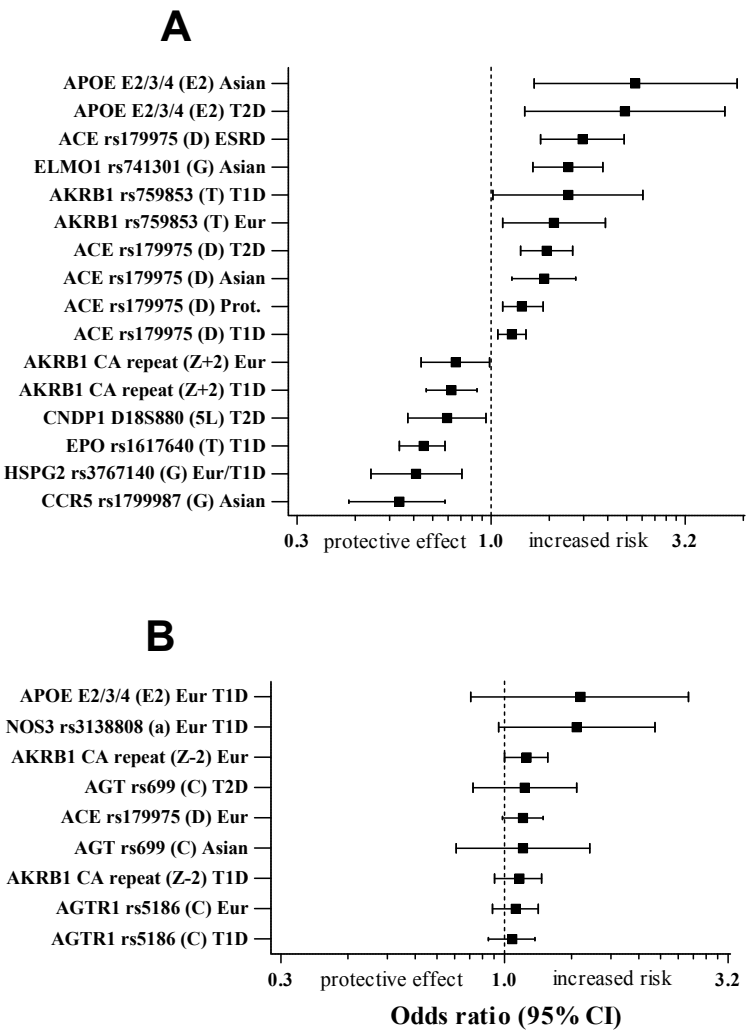

Figure 3. Genetic variants reproducibly associated with diabetic nephropathy in a subgroup. **a** All genetic variants in or near a gene that were reproduced in an independent study and significantly associated with diabetic nephropathy after meta-analysis in a subgroup. **b** All genetic variants in or near a gene that were reproduced in an independent study, but were not significantly associated with diabetic nephropathy after meta-analysis in a subgroup. Parentheses (*y*-axis labelling) contain the allele used in the comparison. The subgroup in which the genetic variant was reproducibly associated with diabetic nephropathy is shown in *y*-axis label as follows: Asian, T2D (type 2 diabetes), ESRD, T1D (type 1 diabetes), Eur (European), Prot. (proteinuria)

Genetic variants involved in the renin–angiotensin system

A variant in *ACE*, rs179975, was the most studied polymorphism in diabetic nephropathy, with 42 studies resulting in a pooled odds ratio of 1.24 (95% CI 1.12-1.37). The association between the deletion of the rs179975 polymorphism and diabetic nephropathy was reproduced in all subgroups. In the meta-analysis, the rs179975 polymorphism was associated with diabetic nephropathy in type 1 diabetes mellitus (OR 1.13 [95% CI 1.04-1.23]), type 2 diabetes mellitus (OR 1.33 [95% CI 1.16-1.52]), ESRD (OR 1.39 [95% CI 1.21-1.60]), proteinuria (OR 1.20 [95% CI 1.07-1.36]) and in the Asian subgroup (OR 1.28 [95% CI 1.10-1.49]), but not in Europeans. Other variants in the renin–angiotensin system that were also widely studied and reproduced, such as the rs699 variant of *AGT* with 21 studies and the rs5186 polymorphism of *AGTR1* with 15 studies, were not associated with diabetic nephropathy in the meta-analysis.

Genetic variants involved in the polyol pathway

The CA repeat and rs759853 in *AKR1B1* were studied in nineteen and nine studies, respectively. The CA repeat has a Z–2 allele thought to lead to an increased risk of diabetic nephropathy and a Z+2 allele thought to have a protective effect. The Z+2 allele and Z–2 allele were both reproducibly associated with diabetic nephropathy, but only the Z–2 allele remained associated in a combined meta-analysis with a pooled odds ratio of 1.12 (95% CI 1.02-1.24). Although reproducibly associated with diabetic nephropathy in 'type 1 diabetes mellitus' and 'European' subgroups, Z–2 was not associated with diabetic nephropathy in the meta-analysis in these subgroups. The Z+2 allele was associated with diabetic nephropathy in the 'type 1 diabetes mellitus' and 'European' subgroups (OR 0.79 [95% CI 0.68-0.92] and OR 0.81 [95% CI 0.66-0.99], respectively). The T allele in SNP rs759853 was associated with risk of diabetic nephropathy in the meta-analysis (OR 1.40 [95% CI 1.13-1.74]) and in the subgroups 'diabetic nephropathy due to type 1 diabetes mellitus' and 'Europeans' (OR 1.58 [95% CI 1.01-2.46] and OR 1.45 [95% CI 1.07-1.97], respectively).

Genetic variants involved in lipid metabolism

Two variants in genes each coding for two different apolipoproteins are reproducibly associated with diabetic nephropathy and remained associated with diabetic nephropathy in the meta-analysis: E2, E3, E4 polymorphism of *APOE* and rs4420638 near *APOC1*. The E2 allele is thought to lead to an increased risk of diabetic nephropathy

and the E4 allele is thought to have a protective effect. Both the E2 and the E4 allele were associated with diabetic nephropathy in the meta-analysis (OR 1.70 [95% CI 1.12-2.58] and OR 0.78 [95% CI 0.62-0.98] respectively). The E2 allele was also reproducibly associated with diabetic nephropathy in the subgroups 'type 2 diabetes mellitus', 'Asians' and 'European/type 1 diabetes mellitus' (all studies investigating Europeans had type 1 diabetes mellitus and vice versa), but only associated with diabetic nephropathy in the meta-analysis in the 'type 2 diabetes mellitus' and 'Asian' subgroups (OR 2.21 [95% CI 1.22-4.00] and OR 2.35 [95% CI 1.29-4.30], respectively). Rs4420638 near the *APOC1* gene was studied in two studies and was associated with diabetic nephropathy in the meta-analysis (OR 1.54 [95% CI 1.29-1.83]). Both studies contained type 1 diabetic nephropathy patients of European descent.

Genetic variants involved in inflammatory cytokines and angiogenesis

Rs1799987 of the *CCR5* (an inflammatory cytokine) gene was only associated with diabetic nephropathy in the Asian subgroup (OR 0.58 [95% CI 0.43-0.76]) consisting of four studies (n=1534), but not in the total group consisting of nine studies (n=5527). For the total group, funnel plot asymmetry was indicated by a significant Begg test.

Two genes involved in angiogenesis, *VEGFA* and *EPO*, each had a variant that was reproducibly associated with diabetic nephropathy. Rs833061 of *VEGFA* was associated with diabetic nephropathy in the meta-analysis in two studies (n=543) containing only type 1 diabetes mellitus patients of European origin (OR 0.48 [95% CI 0.37-0.61]). Rs1617640 of *EPO* was associated with diabetic nephropathy (OR 0.67 [95% CI 0.60-0.76]) in three studies (n=2773), also in the subgroup with type 1 diabetes mellitus patients (OR 0.67 [95% CI 0.58-0.76]).

Genetic variants involved in oxidative stress

Five genetic variants in four genes thought to be related to oxidative stress were reproducibly associated with diabetic nephropathy. The 1/2 polymorphism of *HP* and rs1801282 of *PPARG* were not associated with diabetic nephropathy in the meta-analysis. For *PPARG*, funnel plot asymmetry was observed (p=0.024) suggesting publication bias. The rs3138808 and the rs2070744 variants of *NOS3* were associated with diabetic nephropathy in the meta-analysis (OR 1.31 [95% CI 1.02-1.67] and OR 1.39 [95% CI 1.09-1.78] respectively). The 5L allele of *CNDP1* was associated with diabetic nephropathy only in the 'type 2 diabetes mellitus' subgroup (OR 0.77 [95% CI 0.61-0.97]).

Genetic variants in other pathways

Rs17300539 of *ADIPOQ*, which is believed to mitigate vascular damage, was not associated with diabetic nephropathy in the meta-analysis. Rs841853 of *GLUT1 (also known as SLC2A1)*, coding for a glucose transporter, did not show an association with diabetic nephropathy in eight studies (OR 1.10 [95% CI 0.89-1.35]). Rs1129456 of *GREM1*, which is involved in cell growth and differentiation, was associated with diabetic nephropathy (OR 1.53 [95% CI 1.25-1.89]) in two studies (n=1799). Rs3767140 of *HSPG2*, which is involved in maintenance of glomerular basement membrane electrostatic charge, was also associated with diabetic nephropathy in the meta-analysis (OR 0.72 [95% CI 0.59-0.87]), and additionally with diabetic nephropathy in Europeans with type 1 diabetes mellitus (OR 0.64 [95% CI 0.49-0.84]). Rs13293564 of *UNC13B*, thought to be involved in apoptosis, was associated with diabetic nephropathy in four studies (OR 1.23 [95% CI 1.11-1.35]).

Genetic variants identified by genome-wide association studies

Of the 14 genetic variants found to be reproducibly associated with diabetic nephropathy from genome-wide association studies (GWAS), ten remained associated in the meta-analysis. Rs2268388 of *ACACB*, rs11993333 of *PVT1*, rs39075 near '*CPVL* and *CHN2*', and rs6492208 (not near a gene) were not associated with diabetic nephropathy in the meta-analysis. Another variant near '*CPVL* and *CHN2*', rs39059, was associated with diabetic nephropathy in two studies (n=1705) (OR 0.74 [95% CI 0.64-0.85]). Rs741301 of *ELMO1* was associated with diabetic nephropathy in Asians with type 2 diabetic nephropathy (OR 1.58 [95% CI 1.28-1.94]), but not in combination with a third study of European type 1 diabetes mellitus patients. Rs451041 and rs739401 of *CARS* were associated with diabetic nephropathy in the meta-analysis (OR 1.37 [95% CI 1.21-1.54] and OR 1.32 [95% CI 1.15-1.51] respectively).

Rs1888747 and rs10868025 of *FRMD3* were associated with diabetic nephropathy (OR 0.74 [95% CI 0.65-0.83] and OR 0.72 [95% CI 0.64-0.81] respectively). Another four variants, rs1041466, rs1411766, rs7989848 and rs9521445, which do not lie near a known gene, were associated with diabetic nephropathy in the meta-analysis (OR 1.38 [95% CI 1.21-1.58], OR 1.36 [95% CI 1.20-1.54], OR 1.32 [95% CI 1.16-1.51] and OR 1.35 [95% CI 1.18-1.55] respectively). The variants in *CARS, FRMD3, 'CPVL and CHN2'* and the five variants not near genes were only investigated in European participants with type 1 diabetes mellitus.

Discussion

In this meta-analysis, 21 genetic variants were associated with advanced diabetic nephropathy and three additional variants were associated within specific subgroups. Meta-analysis of several individual genetic variants in relation to diabetic nephropathy has been performed previously, but this is the first complete overview assessing for all genetic variants that are reproducibly associated with the presence of diabetic nephropathy. This information could lead to improved insight into underlying pathogenetic mechanisms. Variants in or near *ACE, AKR1B1* (two variants)*, APOC1, APOE, EPO, NOS3* (two variants)*, HSPG2, VEGFA, FRMD3* (two variants)*, CARS* (two variants)*, 'CPVL and CHN2', UNC13B* and *GREM1*, as well as four variants not near known genes, were associated with diabetic nephropathy. *ELMO1*, *CCR5* and *CNDP1* were associated with diabetic nephropathy in a subgroup ('Asian's and 'type 2 diabetes mellitus' respectively). These results support a role for the following in the pathogenesis of diabetic nephropathy: renin–angiotensin system, polyol pathway, oxidative stress, inflammation, angiogenesis, glomerular filtration barrier defects, apoptosis, and cell growth and differentiation. Functional studies remain to be performed to establish the precise roles of these variants and pathways. Genetic variants initially identified using a genome-wide association approach in and near *FRMD3*, *CARS*, *ELMO1* and *'CPVL and CHN2'* were detected. The exact role of these genetic variants in relation to diabetic nephropathy requires further elucidation; many of these variants identified in GWAS will not prove to be causal.

Our analysis has some limitations. Publication bias is a concern in all meta-analyses. For this study, only published data in journals were used, discarding data published in congresses only. Negative studies are less likely to be published, potentially leading to an overestimation of effects. Moreover, non-significant genetic associations might have been underreported in published articles. Therefore, the effect estimates of the present study should be interpreted with caution, especially in cases where associations were based on small numbers of studies and/or small sample numbers. For example, the rs833061 variant in the *VEGFA* gene shows the strongest protective effect, but was investigated in two studies of moderate size. In these cases, additional studies are necessary to establish true effect sizes. It should also be acknowledged that by selecting only genetic variants that were associated with diabetic nephropathy and for which independent replication was available, genetic variants with smaller effect sizes may

have been missed, an effect that may have proven significant using pooled analyses. By selecting only those genetic variants reproducibly associated with diabetic nephropathy, we have tried to reduce the chances of describing false positive associations.

The studies included in the present analysis showed heterogeneity with respect to ethnicity, study design and phenotypes. For some of the analysis, the clinical heterogeneity was accompanied by statistical heterogeneity with an I^2 statistic of up to 91%. However, there is no fully accepted statistical measure that precisely determines clinical heterogeneity (16). To account for potential heterogeneity, random effects models were performed by default. These models assume that different studies have different true effects. To explore potential heterogeneity due to differences in ethnicity, a meta-regression was performed.

A study worth mentioning, which appeared after our inclusion date, is a paper by Maeda *et al.* (17), in which the authors investigated the variants found in the genome-wide association scan of the Genetics of Kidneys in Diabetes and Diabetes Control and Complications Trial studies (18) in four studies, of which three meet our criteria. We combined the data of Maeda *et al.* with results of the Genetics of Kidneys in Diabetes and Diabetes Control and Complications Trial studies. We found that only the rs451041 of *CARS*, and rs1041466, rs9521455 and rs1411766, which are not near a gene, were associated with diabetic nephropathy. In contrast to the Genetics of Kidneys in Diabetes and Diabetes Control and Complications Trial studies, which investigated Europeans with type 1 diabetes, Maeda *et al.* investigated diabetic nephropathy in type 2 diabetes in an Asian population. Therefore, the lack of association with diabetic nephropathy of the other variants could be explained by this difference.

The identification of diabetic nephropathy susceptibility variants can lead to novel biological insights and improved measures of individual aetiological processes, as indicated previously (19). Individual aetiological processes (personalised medicine) could allow preventive and therapeutic interventions in complex disease to be tailored to individuals on the basis of their genetic profiles. From prediction studies with genetic variants for type 2 diabetes mellitus, it has been shown that 20 established genetic variants in type 2 diabetes mellitus have an AUC of 0.54 (0.5 means no predictive value, 1.0 is perfect prediction), in contrast to the Framingham offspring and Cambridge risk scores (AUC of 0.78 and 0.72, respectively). Interestingly, addition of genetic information to phenotype-based risk models did not improve prediction (20). It is also possible that for diabetic nephropathy the genotypic risk does not exceed the risk contributed by

conventional risk factors (e.g. BMI, age, diabetes mellitus duration), which means that the predictive value of risk variants for diabetic nephropathy would be limited (21). Although genetic prediction and use of personalised medicine in diabetic nephropathy remains a new undertaking, prediction is likely to improve as additional disease variants are detected and replicated (22).

Novel biological insights may lead to development of new therapeutic targets, biomarkers and opportunities for disease prevention. Hypothesis-free approaches, such as GWAS, are most promising in this respect. At present, it seems wise to focus on assessing the relevance of previously detected genetic variants. As common SNPs associated with diabetic nephropathy and detected by GWAS may represent rare genetic variants with large effects, sequencing the regions surrounding highly significant and replicated genomic regions to detect rare variants appears to be reasonable. Follow-up *in vitro* and *in vivo* studies could then assess the functional relevance of these variants in diabetic nephropathy. In summary, our meta-analysis identified 24 genetic variants (in or near 16 different genes) associated with advanced diabetic nephropathy. These genetic variants are likely to represent true associations and further investigations to elucidate their functional relationship in diabetic nephropathy should be pursued.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

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