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CKJ Review

CKJ REVIEW

The genetic map of diabetic nephropathy: evidence from a systematic review and meta-analysis of genetic association studies

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ABSTRACT

Despite the extensive efforts of scientists, the genetic background of diabetic nephropathy (DN) has not yet been clarified. To elucidate the genetic variants that predispose to the development of DN, we conducted a systematic review and meta-analysis of all available genetic association studies (GAS) of DN. We searched in the Human Genome Epidemiology Navigator (HuGE Navigator) and PubMed for available GAS of DN. The threshold for meta-analysis was three studies per genetic variant. The association between genotype distribution and DN was examined using the generalized linear odds ratio (OR_G). For variants with available allele frequencies, the examined model was the allele contrast. The pooled OR was estimated using the DerSimonian and Laird random effects model. The publication bias was assessed with Egger's test. We performed pathway analysis of significant genes with DAVID 6.7. Genetic data of 606 variants located in 228 genes were retrieved from 360 GASs and were synthesized with meta-analytic methods. *ACACB*, *angiotensin I-converting enzyme* (*ACE*), *ADIPOQ*, *AGT*, *AGTR1*, *AKR1B1*, *APOC1*, *APOE*, *ATP1B2*, *ATP2A3*, *CARS*, *CCR5*, *CGNL1*, *Carnosine dipeptidase 1* (*CNDP1*), *CYGB-PRCD*, *EDN1*, *Engulfment and cell motility 1* (*ELMO1*), *ENPP1*, *EPO*, *FLT4*, *FTO*, *GLO1*, *HMGA2*, *IGF2/INS/TH cluster*, *interleukin 1B* (*IL1B*), *IL8*, *IL10*, *KCNQ1*, *KNG*, *LOC101927627*, *Methylenetetrahydrofolate reductase*, *nitric oxide synthase 3* (*NOS3*), *SET domain containing seven, histone lysine methyltransferase* (*SETD7*), *Sirtuin 1* (*SIRT1*), *SLC2A1*, *SLC2A2*, *SLC12A3*, *SLC19A3*, *TCF7L2*, *TGFB1*, *TIMP1*, *TTC39C*, *UNC13B*, *VEGFA*, *WTAPP1*, *WWC1* as well as *XYLT1* and three intergenic polymorphisms showed significant association with DN. Pathway analysis revealed the overrepresentation of six signalling pathways.The significant findings provide further evidence for genetic factors implication in DN offering new perspectives in discovery of new therapies.

Keywords: association, diabetic nephropathy, genetic, meta-analysis, polymorphisms, systematic review

INTRODUCTION

Diabetic nephropathy (DN) has complex aetiology due to synergistic interplay between many factors, modifiable or not. Among them, prominent contribution is attributed to glycaemic and haemodynamic factors, as well as the genetic background [1, 2]. Despite the extensive research, the genetic architecture is poorly understood [3, 4]. Although many genetic variants have

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been implicated in DN susceptibility, the results are not replicable and create inconsistency [4].

Family-based studies through linkage analysis suggest familial clustering in many populations, but this type of method has the potential to detect rare variants with large effects [5–9]. Unlike non-DN and apolipoprotein L1 (APOL1) confirmed susceptibility, in the case of DN such established genetic contribution has not been achieved [10].

Population-based studies, the well-known genetic association studies (GAS), tried to shed light on genetic background on many common diseases [11]. In particular, when the first wave of genome-wide association studies (GWAS) appeared, the researchers were extremely optimistic for the potential of these [12], but even GWAS results have failed to be replicated. In DN, many GWAS have been conducted with promising results, but the burden of DN still reaches alarming proportions [13–24].

In an effort to increase power and enlighten the genetic architecture of DN, meta-analysis of genetic studies promises more convincing evidence. Meta-analysis of genome-wide linkage studies of DN [25] or renal function traits [26] has been performed, indicating suggestive evidence for many cytogenetic locations. We conducted a field synopsis including all the case–control GAS that examine the association between genetic variants and DN.

MATERIALS AND METHODS

Identification and eligibility of relevant studies

To elucidate the contribution of genetic background in development of DN, we conducted a systematic review and meta-analysis of GASs in DN. In the meta-analysis, studies in English recorded in the Human Genome Epidemiology (HuGE) Phenopedia (last update on 3 July 2019) regarding the disease term 'diabetic nephropathies' were included. We also retrieved articles from GWAS in HuGE Publit and the National Human Genome Research Institute (NHGRI) Catalog of Published GWAS (http://www.genome.gov/gwastudies/). We cross-checked manually these findings against those indexed in PubMed using the search terms [('diabetic nephropathy' OR 'diabetic kidney disease' OR 'diabetic end stage renal disease') AND ('genetic association' OR 'gene' OR 'variant' OR 'polymorphism')] (accessed on 7 July 2019). Finally, any meta-analyses and the references of the eligible articles were also screened. We did not request unpublished data from any author.

The included studies should meet the following criteria: (i) they involved cases with persistent micro/macroalbuminuria with or without diabetic retinopathy; (ii) they involved diseased controls with diabetes and normoalbuminuria or normal renal function and/or healthy controls; (iii) they provided full genotypic data, either genotype counts or allele frequencies, excluding the articles with results after having merged genotypes; and (iv) they included human subjects. The diabetes could be either Type I diabetes mellitus (T1DM) or Type II diabetes mellitus (T2DM). No study involving exclusive cases with not persistent microalbuminuria was included.

Studies examining disease progression, phenotype modification, response to treatment or survival were excluded. Case reports, editorials, reviews, non-English articles, unpublished studies as well as studies with other study designs, such as family-based studies, were also excluded. The eligibility of the articles was assessed independently by two investigators (M.T. and E.Z.), the results were compared and any disagreements were resolved by reaching consensus.

Data extraction

From each article, the following information was extracted: first author, year of publication, ethnicity, PubMed ID (PMID) , type of diabetes and the phenotype. For cases and controls, we recorded their number and the selection criteria. With regard to the genotypic data, we extracted, if available, the full genotype counts or allele frequencies.

Data synthesis and analysis

The association between genotypes and DN was examined using the generalized linear odds ratio (OR_G) [27, 28]. For the variants with available allele frequencies, the examined model was the allele-contrast. The threshold for meta-analysis was the presence of three studies. The pooled OR was estimated using DerSimonian and Laird random-effects model [29]. The associations are presented with ORs with corresponding 95% confidence intervals (CIs). We tested for between-study heterogeneity with Cochran's Q statistic (considered statistically significant at $P < 0.10$) and assessed its extent with the I^2 statistic [30, 31]. OR_G was calculated using generalized odds ratio methodology for the analysis and meta-analysis of GAS (ORGGASMA) (http://biomath.med.uth.gr) [27, 28]. Furthermore, we conducted a subgroup analysis regarding the DM type and ethnicity in case of existence of >10 studies per genetic polymorphism.

For each study, we examined if controls confronted with Hardy–Weinberg equilibrium (HWE) predicted genotypes using Fisher's exact test. For studies providing only allele counts, we relied on the authors' assessment of deviations from HWE. We also tested for 'small-study effect' with the Egger test [32].

Pathway analysis

We performed pathway analysis of statistically significant genes with DAVID version 6.7 to identify in the signalling pathways that are overrepresented by the significant genes [33, 34].

RESULTS

Study characteristics

The literature search retrieved 3697 records after removing of duplicates. When an article provided data for different populations, then each population was considered as a different study. Different ethnic descents were categorized as Caucasians, Asians, Africans and mixed. Figure 1 presents a flowchart of retrieved articles and excluded articles with specification of reasons for exclusion. The threshold for a variant in order to be meta-analysed was three studies per variant. Overall, 227 candidate genes and 606 polymorphisms were investigated in 356 articles. The characteristics of each study and their references are shown in Supplementary data, Table S1. The studies were published between 1994 and 2019.

Main meta-analysis results

Tables 1–3 show the statistically significant results of meta-analyses exploring the presence of association between the relevant genetic variants and DN based on genotype counts. An overview of pooled OR_G of the statistically significant variants is shown in Figures 2–5. The statistically significant results of meta-analyses exploring the presence of association between the genetic variants and DN based on allele counts are presented in Table 4. An overview of the meta-analysis results

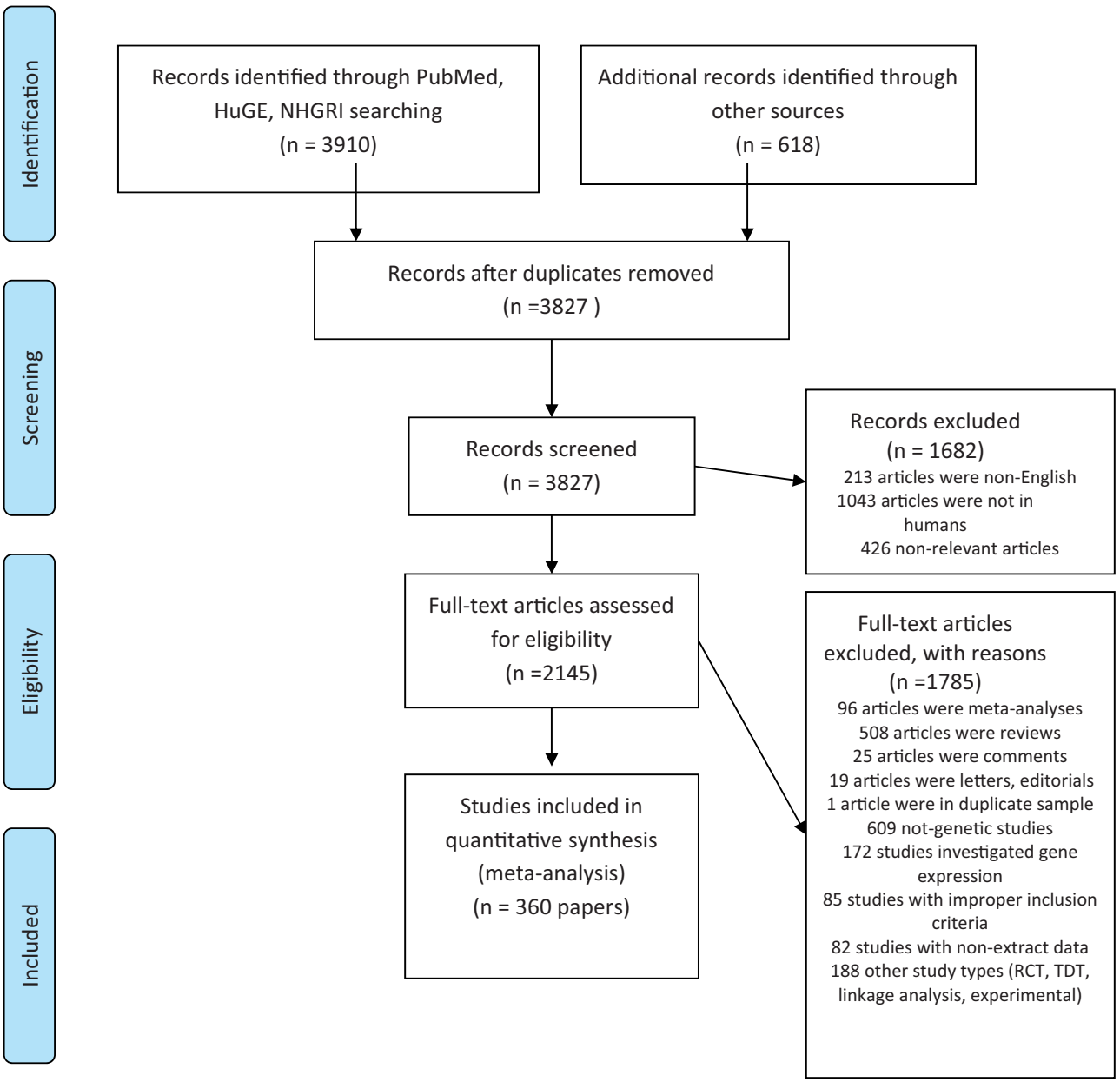

FIGURE 1: Flowchart of retrieved articles with specifications of reasons for exclusion. RCT: randomized controlled trial; TDT: transmission disequilibrium test.

based on allele counts is shown in Figure 5. The meaning of the acronyms of statistically significant genes is shown in Supplementary data, Table S2. The non-significant results from meta-analysis are presented in Supplementary data, Tables S3–S7. Supplementary data, Table S8 summarizes information on the functional implications of the genetic variants associated with DN, indicating whether the gene variants are associated with increased or decreased expression of the gene or increased or decreased activity or levels of the gene product, if this is known.

The pathway analysis was performed for the genes with significant association with DN and revealed the overrepresentation of six signalling pathways: the cytokine–cytokine receptor interaction, the pyruvate metabolism, T2DM, the adipocytokine signalling pathway, the renal cell carcinoma and the renin–angiotensin system pathway. The results of the statistically significant genes are presented based on the relevant signalling pathway (Table 5). Supplementary data, Table S9 illustrates where in the pathway the genes sit.

Genetic variants related to cytokine–cytokine receptor interaction. The polymorphism –59029A/G in *CCR5* showed significant association with DN in comparison between diabetics with normoalbuminuria and cases with DN, with a pooled OR_G of 0.69 (95% CI 0.53–0.91) . This association was also significant in analysis of only studies with controls in HWE.

EPO rs1617640 polymorphism showed significant association with DN in comparison between diabetics without nephropathy and cases with DN with a pooled OR_G of 1.64 (95% CI 1.43–1.89).

Fms related receptor tyrosine kinase 4 (*FLT4*) rs2242221 polymorphism was also associated with DN in analysis based on allele counts, with a pooled OR_G of 1.14 (95% CI 1.01–1.29).

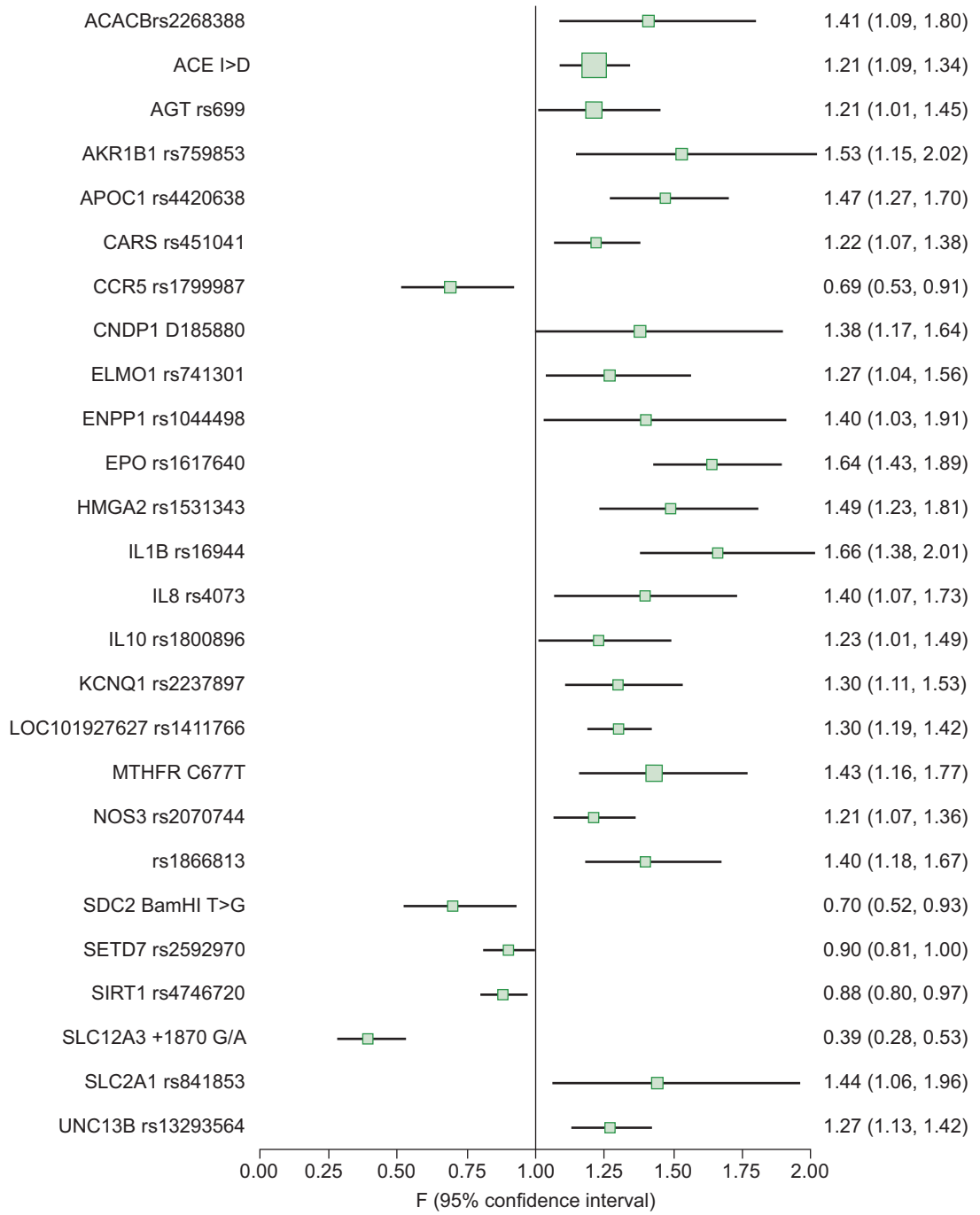

FIGURE 2: Forest plot of diseased controls (diabetics with normoalbuminuria) and cases (diabetics with diabetic nephropathy) displaying only significant results based on genotype counts.

The polymorphism –511C/T in *interleukin 1B* (*IL1B*) showed significant association with DN with an OR_G of 1.66 (95% CI 1.38–2.01).

The polymorphism –251T/A in *IL10* in analysis between diabetics with normoalbuminuria and cases with DN was also associated with DN, with a pooled OR_G of 1.40 (95% CI 1.07–1.73).

The polymorphism T869C in *TGFB1* also showed significant association with DN in both analysis between three groups and in analysis between healthy controls and cases with DN, with a pooled OR_G of 1.36 (95% CI 1.08–1.70) and OR_G of 1.73 (95% CI 1.46–2.04), respectively.

Rs2146323 polymorphism in *vascular endothelial growth factor A* (*VEGFA*) was significantly associated with DN, with a pooled OR of 0.85 (95% CI 0.76–0.95) when diseased controls were compared with cases with DN with the allele contrast model.

Genetic variants related with pyruvate metabolism. Two polymorphisms in the *acetyl-CoA carboxylase beta* (*ACACB*) gene, rs2268388 and rs5186, showed significant association with DN when diabetics without DN were compared with cases with DN, with a pooled OR_G of 1.41 (95% CI 1.09–1.80) and 1.59 (95% CI 1.28–1.98),

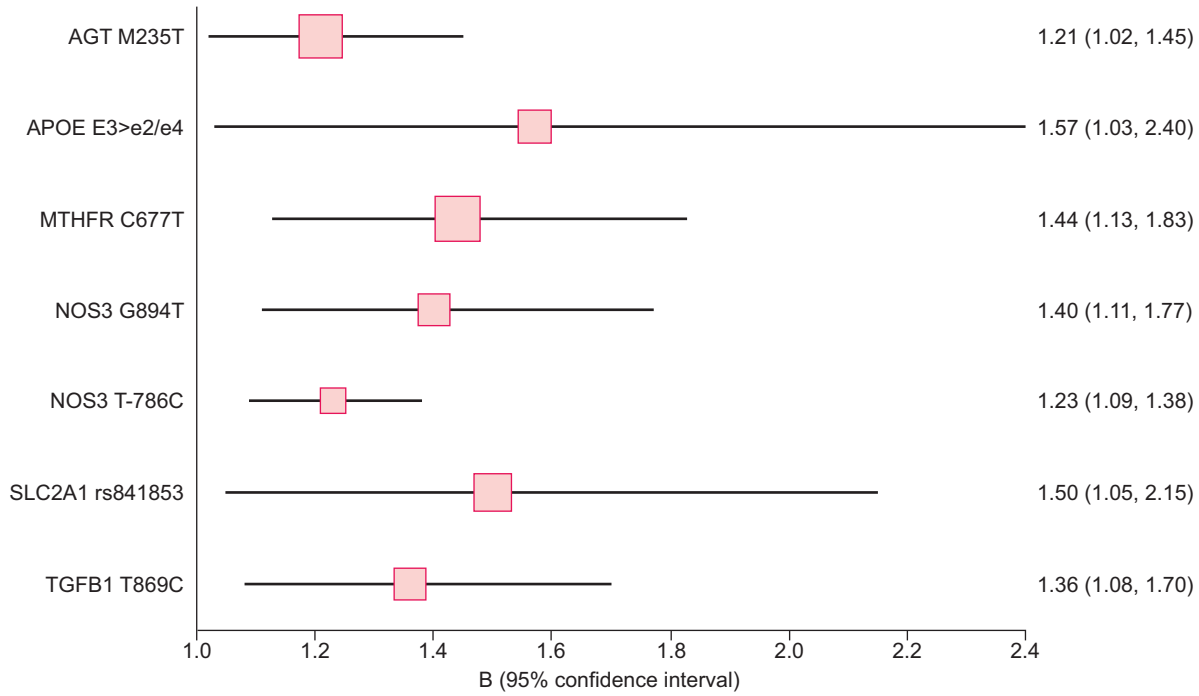

FIGURE 3: Forest plot of healthy controls versus diseased controls (diabetics with normoalbuminuria) versus cases (diabetics with diabetic nephropathy) displaying only significant results based on genotype counts.

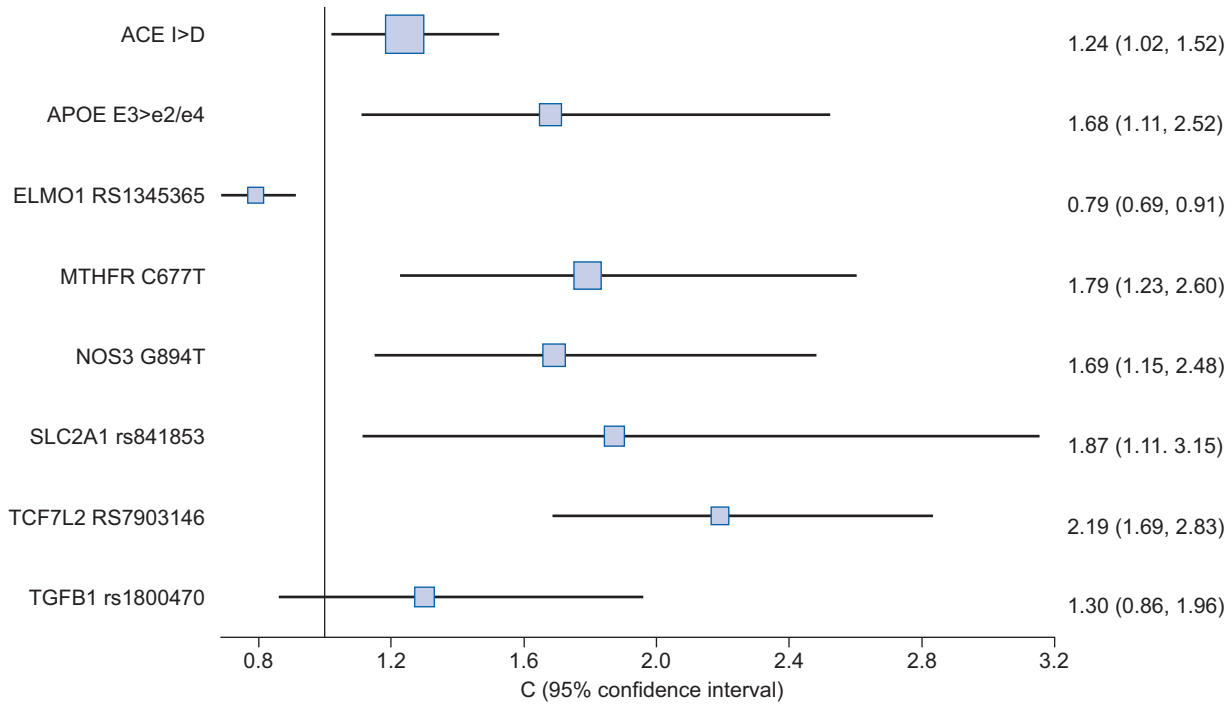

FIGURE 4: Forest plot of healthy controls and cases (diabetics with diabetic nephropathy) displaying only significant results based on genotype counts.

respectively. The aforementioned associations were also significant in meta-analysis with studies in HWE.

In a comparison of diseased controls versus cases, the *AKR1B1* rs759853 variant was significantly associated with DN, with a pooled OR_G of 1.53 (95% CI 1.15–2.02). The association was also significant in meta-analysis of studies in HWE (1.51, 95% CI 1.08–2.10).

Rs7769206 in *GLO1* showed a significant association with DN in comparison between diabetics with normoalbuminuria and cases with DN, with a pooled OR_G of 1.22 (95% CI 1.02–1.47).

Genetic variants related with T2DM. The polymorphism –11391G/A in *ADIPOQ* showed a significant association with DN in comparison between diabetics with normoalbuminuria

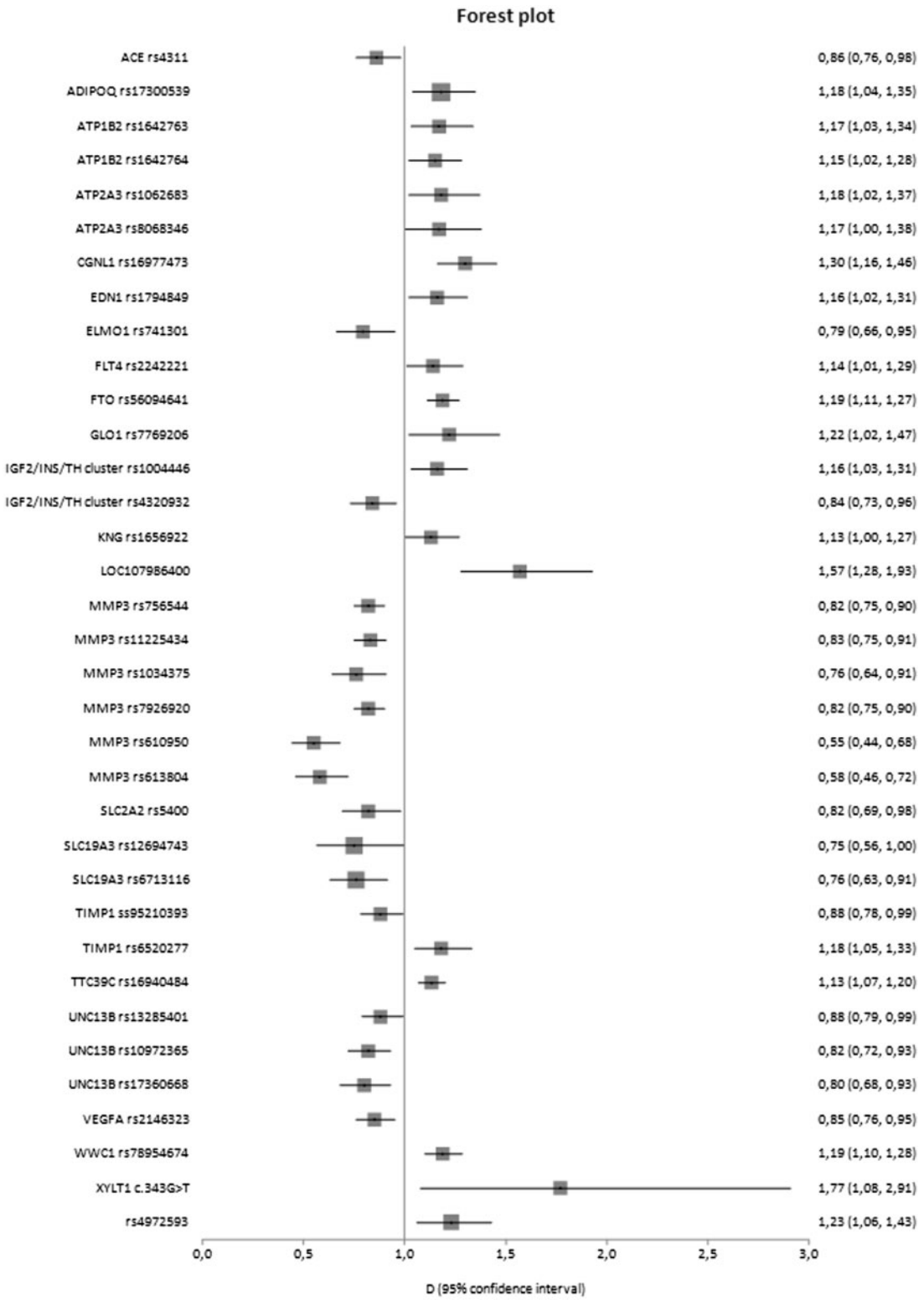

FIGURE 5: Forest plot of diseased controls (diabetics with normoalbuminuria) and cases (diabetics with diabetic nephropathy) displaying only significant results based on allele counts.

and cases with DN, with a pooled OR_G of 1.18 (95% CI 1.04–1.35).

The polymorphisms rs10004446 and rs4320932 in the *IGF2/INS/TH* cluster showed a significant association with DN in comparison between diabetics with normoalbuminuria and cases with DN, with a pooled OR_G of 1.16 (95% CI 1.03–1.31) and OR_G of 0.84 (95% CI 0.73–0.96), respectively.

The polymorphism rs5400 in *SLC2A2* was also significant when diabetics with normoalbuminuria and cases with DN were compared, with a pooled OR_G of 0.82 (95% CI 0.69–0.98).

Table 1. Statistically significant results from meta-analysis between diseased controls (diabetics with normoalbuminuria) versus cases (diabetics with diabetic nephropathy) based on genotype counts

Gene	Variant	RS	Studies (*n*)	Cases/controls (*n*)	RE OR_G (95% CI)	I^2 (%)	P_Q	P_E
ACACB	(C > T)	rs2268388	10	3222/2881	1.41 (1.09–1.80)	81.65	0	0.3
	All in HWE		7	2424/2190	1.30 (1.02–1.65)	72.54	0	0.48
ACE	I > D		65	10787/10404	1.21 (1.09–1.34)	74.61	0	0.70
	All in HWE	I > D	55	8733/8267	1.26 (1.14–1.39)	63.85	0	0.54
AGT	M235T	rs699	26	5015/5253	1.21 (1.01–1.45)	82,45	0,00	0.84
	All in HWE		19	3181/3655	1.09 (0.92–1.31)	72.76	0.00	0.95
AKR1B1	C-106T	rs759853	7	1149/1068	1.53 (1.15–2.02)	65.11	0.01	0.3
	All in HWE		6	1064/922	1.51 (1.08–2.10)	70.38	0	0.33
APOC1	C > T	rs4420638	3	1526/1620	1.47 (1.27–1.70)	0	0.61	0.21
CARS	(G > A)	rs451041	5	3061/3170	1.22 (1.07–1.38)	47.41	0.11	0.06
	All in HWE		4	2571/2230	1.25 (1.07–1.45)	50.50	0.11	0.06
CCR5	−59029 A > G	rs1799987	8	2125/2127	0.69 (0.53–0.91)	83.21	0.00	0.04
		All in HWE	6	1789/1780	0.79 (0.60–1.03)	80.27	0.00	0.07
CNDP1	$[CTG]_5 > [CTG]_{6/7}$	D18S880	11	4064/7318	1.38 (1.17–1.64)	64.78	0.002	0.01
	All in HWE		10	3184/3316	1.47 (1.19–1.82)	66.46	0.001	0.002
ELMO1		rs741301	5	1072/984	1.27 (1.04–1.56)	32.92	0.20	0.83
	All in HWE		4		1.23 (0.91–1.66)	44.24	0.15	0.40
ENPP1	K173Q (A > C)	rs1044498	4	744/982	1.40 (1.03–1.91)	62.33	0.05	0.21
	All in HWE		3		1.27 (0.90–1.81)	59.93	0.08	0.25
EPO	G > T	rs1617640	3	1618/954	1.64 (1.43–1.89)	0.00	0.78	0.03
HMGA2	(G/C)	rs1531343	3	1233/2125	1.49 (1.23–1.81)	0	0.41	0.45
IL1B	−511C/T	rs16944	3	774/665	1.66 (1.38–2.01)	0	0.86	
IL8	−251 T > A	rs4073	5	661/703	1.40 (1.07–1.73)	36.43	0.18	0.49
	All in HWE		4	611/565	1.28 (0.98–1.67)	38.13	0.18	0.26
IL10	−1082 A > G	rs1800896	4	677/761	1.23 (1.01–1.49)	0	0.56	0.63
		All in HWE	2	610/690	1.25 (1.02–1.53)	0	0.62	NA
KCNQ1		rs2237897	4	1539/2208	1.30 (1.11–1.53)	30.60	0.23	0.12
LOC101927627	G > A	rs1411766	6	3780/3561	1.30 (1.19–1.42)	0	0.64	0.77
	All in HWE		5		1.31 (1.19–1.45)	0	0.53	0.82
MTHFR	C677T		26	4246/4380	1.43 (1.16–1.77)	83.91	0	0.49
	All in HWE		21	3163/3085	1.28 (1.03–1.59)	78.12	0	0.51
NOS3	T-786C	rs2070744	7	1934/1710	1.21 (1.07–1.36)	0.56	0.42	0.49
	All in HWE		5	1672/1418	1.22 (1.04–1.44)	33.45	0.20	0.17
rs1866813			5	1798/1831	1.40 (1.18–1.67)	26.09	0.25	0.02
SDC2	BamHI T > G		3	485/231	0.70 (0.52–0.93)	0	0.45	0.14
SETD7		rs2592970	3	1939/2222	0.90 (0.81–0.997)	0	0.44	0.3
SIRT1		rs4746720	6	2785/2484	0.88 (0.80–0.97)	0	0.55	0.08
		All in HWE	6					
SLC12A3	+1870 G/A		3	1070/650	0.39 (0.28–0.53)	0	0.73	0.01
	All in HWE		2	558/334	0.36 (0.23–0.57)	0	0.52	NA
SLC2A1	XbaI(+) > XbaI(−)	rs841853	10	1438/1331	1.44 (1.06–1.96)	78.03	<0.001	0.13
	All in HWE		7	938/840	1.71 (1.19–2.45)	75.80	<0.001	0.30
UNC13B		rs13293564	4	1573/1910	1.27 (1.13–1.42)	0	0.54	0.08

RS: SNP identifier, RE OR_G: random effects odds ratio generalized, I^2: I^2 statistic, NA: non applicable, P_Q: P-value from heterogeneity testing, P_E: P-value from Egger's test.

Genetic variants related with adipocytokine signalling pathway. Except *ACACB* and *adiponectin* (*ADIPOQ*) variants significant association, *SLC2A1* rs841853 variant was also significantly associated with DN, with a pooled OR_G of 1.44 (95% CI 1.06–1.96). This association remained significant in meta-analysis with the seven studies in HWE (1.71, 95% CI 1.19–2.45). Rs841853 was also significantly associated with DN in meta-analysis with three groups and between healthy controls and cases with DN, with a pooled OR_G of 1.50 (95% CI 1.05–2.15) and 1.87 (95% CI 1.11–3.15).

Genetic variants related with renal cell carcinoma. In this pathway, *SLC2A1*, *TGFB1* and *VEGFA* are involved, which were mentioned above.

Genetic variants related with renin–angiotensin system. In diseased controls versus cases, the deletion of the polymorphism rs179975 in *angiotensin I-converting enzyme* (*ACE*) was associated with DN, with a pooled OR_G of 1.21 (95% CI 1.09–1.34) after meta-analysis conducted in 65 studies. The association was also significant in sensitivity analysis of 55 studies in HWE with an OR_G of 1.26 (95% CI 1.14–1.39). The polymorphism rs179975 was also associated with DN, with an OR_G of 1.24 (95% CI 1.02–1.52) when healthy controls were compared with cases with DN.

Another gene implicated in the renin–angiotensin–aldosterone system with significant results in a meta-analysis of 26 studies is *AGT*, with an OR_G of 1.21 (95% CI 1.01–1.45). The variant M235T (rs699) was also associated with DN in comparison between healthy controls versus diabetics without nephropathy versus cases with DN, with a pooled OR_G of 1.21 (95% CI 1.02–1.45).

Table 2. Statistically significant results from meta-analysis between healthy controls versus diseased controls (diabetics with normoalbuminuria) versus cases (diabetics with diabetic nephropathy) based on genotype counts

Gene	Variant	RS	Studies (*n*)	Cases/diseased controls/healthy (*n*)	RE OR_G (95% CI)	I^2 (%)	P_Q	P_E
AGT	M235T	rs699	9	1156/1350/1243	1.21 (1.02–1.45)	65.91	0	0.11
APOE	E3 > e2/e4		6	743/571/1171	1.57 (1.03–2.40)	86.26	0	0.4
MTHFR	C677T		10	1345/1306/1903	1.44 (1.13–1.83)	84.45	0	0.3
	All in HWE		6		1.14 (0.98–1.32)	26.51	0.24	0.49
NOS3	G894T	rs1799983	6	1152/811/117	1.26 (0.94–1.70)	77.82	<0.001	0.95
	G894T	rs1799983	5	1085/775/1027	1.40 (1.11–1.77)	58.93	0.05	0.16
	T-786C	rs2070744	3		1.23 (1.09–1.38)	0	0.76	0.83
	All in HWE		2		1.22 (1.08–1.38)	0	0.59	–
SLC2A1	XbaI(+) > XbaI(−)	rs841853	7		1.50 (1.05–2.15)	89.18	0	0.49
TGFB1	T869C	rs1800470	5	770/787/1332	1.36 (1.08–1.70)	73.19	0	0.24
		All in HWE	4	706/727/1103	1.47 (1.24–1.75)	45.66	0.14	0.15

RS: SNP identifier, RE OR_G: random effects odds ratio generalized, I^2: I^2 statistic, NA:nonapplicable,P_Q: *P*-value from heterogeneity testing, P_E: *P*-value from Egger's test.

Table 3. Statistically significant results from meta-analysis between healthy controls versus cases (diabetics with diabetic nephropathy) based on genotype counts

Gene	Variant	RS	Studies (*n*)	Cases/controls (*n*)	RE OR_G (95% CI)	I^2 (%)	P_Q	P_E
ACE	I > D		30	3690/4927	1.24 (1.02–1.52)	83.20	0.00	0.03
	All in HWE	I > D	29	3283/4695	1.26 (1.02–1.55)	82.87	0.00	0.01
APOE	E3 > e2/e4		7	1109/1371	1.68 (1.11–2.52)	73.91	0	0.14
ELMO1		rs1345365	3	1204/1241	0.79 (0.69–0.91)	0	0.92	0.55
	All in HWE		3					
MTHFR	C677T		14	1652/2603	1.79 (1.23–2.60)	88.10	0	0.91
	All in HWE		8	847/1295	1.41 (0.94–2.12)	80.38	0	0.36
NOS3	G894T	rs1799983	9	1941/2222	1.69 (1.15–2.48)	85.21	0	0.18
		All in HWE	7	1826/1852	1.84 (1.26–2.7)	83.62	0	0.04
SLC2A1	XbaI(+) > XbaI(−)	rs841853	7		1.87 (1.11–3.15)	89.74	0	0.89
	All in HWE		7					
TCF7L2	C > T	rs7903146	4	1262/2526	2.19 (1.69–2.83)	70.75	0.02	0.44
		All in HWE	3	685/1930	2.46 (2.00–3.01)	27.03	0.25	0.43
TGFB1	T869C	rs1800470	6	814/1450	1.30 (0.86–1.96)	83.64	0	0.18
		All in HWE	4	706/1103	1.73 (1.46–2.04)	0	0.41	0.21

RS: SNP identifier, RE OR_G: random effects odds ratio generalized, I^2: I^2 statistic, NA:nonapplicable,P_Q: *P*-value from heterogeneity testing, P_E: *P*-value from Egger's test.

Some genes were not classified in signalling pathways and thus, these genes are discussed based on their biologic role and function.

Genetic variants involved in lipid metabolism. The polymorphism rs4420638 of the *APOC1* gene was significantly associated with DN, with a pooled OR_G of 1.47 (95% CI 1.27–1.70) in a comparison of diabetics without nephropathy and cases with DN. In a comparison of healthy controls versus diseased controls versus cases, and comparison of healthy controls versus cases with DN, variant E2 and E4 was significantly associated with DN, with a pooled OR_G of 1.57 (95% CI 1.03–2.40) and 1.68 (95% CI 1.11–2.52), respectively.

Genetic variants involved in endothelial function. The polymorphism –786T/C in *nitric oxide synthase 3* (*NOS3*) gene was significantly associated with DN with a pooled OR_G of 1.21 (95% CI 1.07–1.36), and this association was also significant in meta-analysis of only studies in HWE. The polymorphism –786T/C in the *NOS3* gene also showed a significant association with DN in a comparison of healthy controls versus diabetics without DN versus cases with DN, with an OR_G of 1.23 (95% CI 1.09–1.38), which was also significant in meta-analysis with only studies in HWE.

Another polymorphism in *NOS3* gene, G894T, was significantly associated with DN both in comparison with three groups and between healthy controls versus cases, with DN with a pooled OR_G of 1.40 (95% CI 1.11–1.77) and 1.69 (95% CI1.15–2.48), respectively.

Genetic variants involved in epigenetic procedures. *SET domain containing seven, histone lysine methyltransferase* (*SETD7*) rs2592970 polymorphism showed a significant protective association with DN with a pooled OR_G of 0.90 (95% CI 0.81–0.997).

Methylenetetrahydrofolate reductase (*MTHFR*) is critical for production of S-adenosyl-l-methionine, the principal methyl donor. The C677T polymorphism in *MTHFR* gene showed a significant association with DN, with a pooled OR_G of 1.45 (95% CI 1.16–1.82). This significance was also significant in meta-analysis of only studies in HWE. C677T was also significantly associated with DN in comparisons of three groups and between comparison of healthy controls versus cases with DN, with a pooled OR_G of 1.44 (95% CI 1.13–1.83) and 1.68 (1.15–2.47), respectively.

Table 4. Statistically significant results from meta-analysis between diseased controls (diabetics with normoalbuminuria) versus cases (diabetics with diabetic nephropathy) based on allele counts

Gene	Variant	RS	Studies (*n*)	Cases/controls (*n*)	RE OR (95% CI)	I^2 (%)	P	PE
		rs4972593	4	1582/3499	1.23 (1.06–1.43)	27.22	0.25	0.07
ACE	T8968C	rs4311	3	1042/1123	0.86 (0.76–0.98)	14.88	0.31	0.27
ADIPOQ	−11391G > A	rs17300539	6	2629/3039	1.18 (1.04–1.35)	0	0.46	0.38
ATP1B2		rs1642763	3	1176/1323	1.17 (1.03–1.34)	0	0.45	0.14
		rs1642764	3	1176/1323	1.15 (1.02–1.28)	0	0.81	0.07
ATP2A3		rs1062683	3	1176/1323	1.18 (1.02–1.37)	0	0.54	0.3
		rs8068346	3	1176/1323	1.17 (1.00–1.38)	0	0.39	0.3
CGNL1		rs16977473	3	1176/1323	1.299 (1.161–1455)	0	0.399	0.51
CYGB. PRCD		rs895157	3	1176/1323	1.214 (1.114–1.323)	0	0.726	0.68
EDN1		rs1794849	3	1176/1323	1.16 (1.02–1.31)	0	0.62	0.08
ELMO1	A > G	rs741301	3	1526/1563	0.793 (0.661–0.951)	56.19	0.102	0.09
FLT4		rs2242221	3	1176/1323	1.14 (1.01–1.29)	0	0.38	0.43
FTO		rs56094641	3	1176/1323	1.187 (1.110–1.269)	0	0.783	0.51
GLO1		rs7769206	3	1176/1323	1.22 (1.02–1.47)	0	0.73	0.07
IGF2/INS/TH cluster		rs1004446	3	1176/1323	1.16 (1.03–1.31)	0	0.49	0.22
		rs4320932	3	1176/1323	0.84 (0.73–0.96)	0	0.43	0.06
KNG		rs1656922	3	1057/1127	1.13 (1.003–1.27)	0	0.90	0.36
LOC107986400			3		1.570 (1.277–1.929)	0	0.614	0.42
–	–	rs11225445	3	1640/1770	1.26 (1.14–1.39)	0.27		0.27
–		rs610950	3	1640/1770	0.55 (0.44–0.68)	0.36		0.36
–		rs613804	3	1640/1770	0.58 (0.46–0.72)	0.37		0.37
SLC2A2		rs5400	3	1057/1127	0.82 (0.69–0.98)	0	0.54	0
SLC19A3		rs12694743	5	2086/603	0.750 (0.564–0.997)	37.91	0.169	0.21
		rs6713116	5	2086/602	0.759 (0.631–0.914)	0	0.498	0.23
TIMP1		ss95210393	3	1176/1323	0.88 (0.78–0.99)	0	0.73	0.46
		rs6520277	3	1176/1323	1.18 (1.05–1.33)	10.75	0.33	0.3
TTC39C		rs16940484	3	1176/1323	1.132 (1.066–1.202)	0	0.988	0.25
UNC13B		rs13285401	3	1176/1323	0.88 (0.79–0.99)	0	0.49	0.1
		rs10972365	3	1176/1323	0.82 (0.72–0.93)	0	0.46	0.41
		rs17360668	3	1176/1323	0.80 (0.68–0.93)	24.65	0.27	0.45
VEGFA	C > A	rs2146323	3	1176/1323	0.85 (0.76–0.95)	0.2		0.2
WTAPP1		rs756544	3	1640/1770	0.82 (0.75–0.90)	0.46		0.46
		rs11225434	3	1640/1770	0.83 (0.75–0.91)	0.46		0.46
		rs1034375	3	3280/3540	0.76 (0.64–0.91)	0.38		0.38
		rs7926920	3	820/885	0.82 (0.75–0.90)	0.46		0.46
WWC1		rs78954674	3	1176/1323	1.188 (1.100–1.284)	0	0.597	0.25
XYLT1	c.343G > T		3	501/485	1.77 (1.08–2.91)	0	0.682	0.49

RS: SNP identifier, RE OR_G: random effects odds ratio generalized, I^2: I^2 statistic, NA:nonapplicable,P_Q: P-value from heterogeneity testing, P_E: P-value from Egger's test.

Table 5. Results from pathway analysis of statistically significant genes

Category	Term	Genes	Count	%	P-value	Benjamini
KEGG_PATHWAY	Cytokine–cytokine receptor interaction	*CCR5*, *EPO*, *FLT4*, *IL1B*, *IL10*, *TGFB1*, *VEGFA*	7	1.2	4.4E-3	3.0E-1
KEGG_PATHWAY	Pyruvate metabolism	*ACACB*, *AKR1B1*, *GLO1*	3	0.5	2.4E-2	6.3E-1
KEGG_PATHWAY	T2DM	*ADIPOQ*, *IFG2*, *INS*, *SLC2A2*	3	0.5	3.3E-2	5.9E-1
KEGG_PATHWAY	Adipocytokine signalling pathway	*ACACB*, *ADIPOQ*, *SLC2A1*	3	0.5	6.2E-2	7.3E-1
KEGG_PATHWAY	Renal cell carcinoma	*SLC2A1*, *TGFB1*, *VEGFA*	3	0.5	6.7E-2	6.8E-1
KEGG_PATHWAY	Renin–angiotensin system	*ACE*, *AGT*	2	0.4	9.9E-2	7.5E-1

Sirtuin 1 (*SIRT1*), a NAD+-dependent deacetylase, and more specifically its variant, rs4746720, showed significant association with DN, with a pooled OR_G of 0.89 (95% CI 0.81–0.99).

Genetic variants identified by GWAS. Rs451041 in the *CARS* gene was significantly associated with DN with a pooled OR_G of 1.22 (95% CI 1.07–1.38). This association was also significant in meta-analysis of only studies in HWE.

Carnosine dipeptidase 1 (*CNDP1*) D18S880 variant was also associated with DN with a pooled OR_G of 1.38 (95% CI 1.17–1.64), and this association was also significant in meta-analysis with studies in HWE. The *Engulfment and cell motility 1* (*ELMO1*) rs741301 polymorphism was significantly associated with DN with a pooled OR_G of 1.27 (95% CI 1.04–1.56). *Solute carrier family 12 member 3* (*SLC12A3*) 1870G/A showed a significant protective effect against DN, with a pooled OR_G of 0.39 (95% CI 0.28–0.53).

Other genes. K173Q in *ENPP1*, rs13293564 in *UNC13B*, rs1531343 in *HMGA2, SDC2* BamHI T/G polymorphism, rs7903146 in *TCF7L2*, *T869C* in *TGFB1*, rs2237897 in *KCNQ*, rs1866813 and two loci, *LOC101927627* and *LOC105370358*, showed significant association with DN. Regarding the allele contrast model, significant associations were observed for *ACE*, *ADIPOQ*, *ATP1B2*, *ATP2A3*, *CGNL1*, *CYGB*, *PRCD*, *EDN1*, *ELMO1*, *FTO*, *KNG*, *LOC107986400*, *WTAPP1*, *SLC19A3*, *TIMP1*, *TTC39C*, *UNC13B*, *WWC1* and *XYLT1*. Genes *ACE*, *ELMO1* and *UNC13B* were reproducibly associated in meta-analysis based on genotype counts.

Subgroup analysis

When there were >10 studies per variant, a subgroup analysis was conducted regarding the type of diabetes and the ethnicity of the participants. More specifically, subgroup analyses were conducted for variants in seven genes including *ACE*, *AGT*, *AGTR1*, *APOE*, *MTHFR*, *NOS3* and *TGFB1* (Table 6). The majority of the variants were reproducibly associated with DN in more than one subgroup analysis.

The A1166C polymorphism of the *AGTR1* gene, while not statistically significant in the main analysis, was revealed as significant in the subgroup analysis of the Asians and in the subgroup analysis of T2DM, as well as polymorphism T869C of the *TGFB1* gene in the case of genotypic analysis in diabetics versus cases with DN where in the main analysis there was no significance, in the subgroup analyses of Asians and T2DM was statistically significant.

DISCUSSION

To the best of our knowledge, the present systematic review and meta-analysis are the most comprehensive in the field of DN as it examines for the first time such a large number of genes and genetic polymorphisms, and in particular synthesizes data of 228 genes (606 polymorphisms). Sixty-six genetic polymorphisms have been shown to be associated with DN. The aforementioned polymorphisms are harboured in 51 genes, while one is not close to any known gene, however this does not mean that it is of secondary importance. The specific polymorphisms belong to the following 51 genetic loci: *ACACB*, *ACE*, *ADIPOQ*, *AGT*, *AGTR1*, *AKR1B1*, *APOC1*, *APOE*, *ATP1B2*, *ATP2A3*, CARS, CCR5, *CGNL1*, *CNDP1*, *CYGB-PRCD*, *EDN1*, *ELMO1*, *ENPP1*, *EPO*, *FLT4*, *FTO*, *GLO1*, *HMGA2*, *IGF2/INS/TH* cluster, *IL1B*, *IL8*, *IL10*, *KCNQ1*, *KNG*, *LOC101927627*, *MTHFR*, *NOS3*, *SDC2*, *SETD7*, *SIRT1*, *SLC2A1*, *SLC2A2*, *SLC12A3*, *SLC19A3*, *TCF7L2*, *TGFB1*, *TIMP1*, *TTC39C*, *UNC13B*, *VEGFA*, *WTAPP1*, *WWC1* as well as *XYLT1* and three intergenic polymorphisms. Of the above genes, *KNG* and *SETD7* genes proved marginally statistically significant.

The present systematic review and meta-analysis confirmed the statistical significance of *ACE*, *AKR1B1*, *APOC1*, *APOE*, *CARS*, *CCR5*, *CNDP1*, *ELMO1*, *EPO*, *NOS3*, *UNC13B* and *VEGFA*, which showed significance in meta-analyses [35, 36]. However, the present meta-analysis provides novel statistical significance in or near *ADRB3*, *ATP1B2*, *ATP2A3*, *CGNL1*, *CYGB-PRCD*, *FLT4*, *LOC101927627*, *TIMP1*, *TTC39C*, *WTAPP1* and *WWC1* genetic loci. Some of the novel findings are harboured in cytogenetic locations, which were revealed significant in a meta-analysis of genome-wide linkage studies, such as locations on chromosome 5 [25].

The pathway analysis of significant genes revealed overrepresentation of six signalling pathways: cytokine–cytokine receptor interaction, pyruvate metabolism, T2DM, adipocytokine signalling pathway, renal cell carcinoma and the renin–angiotensin system.

Taking into consideration the aforementioned pathways and the classification of genes based on biological role indicated the important role played by the renin–angiotensin system [37], angiogenesis and erythropoiesis [38], lipid metabolism [39], polyol pathway [40], inflammatory mechanisms [36, 41], oxidative stress [42], endothelium function [43] and extracellular matrix degradation [44], as well as epigenetic mechanisms [45] and glucose transport [46, 47]. Functional studies remain necessary to confirm the involvement of these mechanisms, in order to clarify the exact role of these polymorphisms and pathways in DN. From the genetic polymorphisms of the *FRMD3*, *CARS*, *ELMO1*, *CPVL* and *CHN2* genes, which were first detected in large genetic correlation studies, only *CARS* and *ELMO1* genes remained statistically significant after the meta-analysis. The exact role of these genes requires further elucidation, but certainly many of the polymorphisms that GWAS have demonstrated will prove to be not really responsible.

Subgroup analyses were performed purely in order to detect statistical significance for a particular polymorphism in a particular environment, either T1DM or T2DM, or Caucasians or Asians. One finding that is worth mentioning is that genes that have been statistically significant in subgroup analyses in the Caucasians have also emerged as significant in subgroup analyses of T1DM (*MTHFR* and *AGT*), while significant genes in subanalysis of Asians were significant and in subgroup analyses by T2DM (*ACE*, *AGTR1*, *MTHFR* and *TGFB1*). This pattern of significance between Caucasians and T1DM, as well as between Asians and T2DM, should be investigated in further studies.

A difference of the present meta-analysis compared with other meta-analyses is the fact that three types of comparisons were made: healthy controls versus cases with DN, diabetic controls versus cases with DN, as well as healthy controls versus diabetic controls versus cases with DN, in order to separate genes whose aggravating role is independent of the presence of diabetic milieu. This information may highlight unknown aspects of the pathophysiology of the disease in the hope of leading the scientific community to discover new therapies that target pathways common to both T1DM and T2DM nephropathy. The field synopsis has some limitations, one of which is the publication bias. In the present field synopsis, we included only English articles published in scientific journals. In addition, in most meta-analyses, a small number of studies were included, so the results should be interpreted with caution. The majority of included studies also had insufficient statistical power in order to identify modest genetic effect which is believed to result from common variants.

The studies included in the meta-analyses differed with regard to the ethnicity of the individuals, the type of diabetes and the clinical phenotype regarding the presence or not of persistent albuminuria. Other studies included subjects with persistent proteinuria, and some included cases with persistent microalbuminuria. However, no study involving exclusively cases with not persistent microalbuminuria was included, so that there is no underestimation of the genetic effect since not persistent albuminuria is a potentially reversible condition. For this reason, the model of random effects was used in the meta-analysis in which the variability of the determinant result is due to both the variability of each study due to the fact that samples are used rather than source populations and variability between different studies. In order to take into account heterogeneity due to different origins and types of diabetes, the relevant subgroup analyses were performed.

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Table 6. Subgroup analysis results based on type of diabetes and ethnicity

	Gene	Variant	RS	Studies (*n*)	Cases/controls (*n*)	RE OR_G (95% CI)	I^2 (%)	P_Q
Disease controls–cases								
ACE	All	I > D		65	10787/10404	1.21 (1.09–1.34)	74.61	0
	All in HWE			55	8733/8267	1.26 (1.14–1.39)	63.85	0
	Caucasians			25	4176/4008	1.1 (0.99–1.22)	38.92	0.03
	Asians			35	5228/5013	1.24 (1.05–1.45)	78.59	<0.001
	T1DM			19	2852/2973	1.1 (0.96–1.25)	43.68	0.02
	T2DM			46	7935/7431	1.25 (1.10–1.43)	79.10	<0.001
	Three groups							
ACE	All	I > D		21	2973/2622/3668	1.12 (0.94–1.34)	88.33	0
	All in HWE			20	2566/2437/3436	1.13 (0.94–1.37)	88.32	0
	Caucasians			7	865/691/1075	1.03 (0.82–1.3)	73.52	0
	Asians			12	1394/1429/1745	1.06 (0.91–1.23)	68.48	0
	T1DM			6	815/575/1024	1.01 (0.78–1.31)	77.09	0
	T2DM			15	2158/2047/2644	1.15 (0.92–1.44)	89.81	0
					Healthy cases			
ACE	All	I > D		30	3690/4927	1.24 (1.02–1.52)	83.2	0
	All in HWE			29	3283/4695	1.26 (1.02–1.55)	82.87	0
	Caucasians			10	1051/1565	1.14 (0.92–1.43)	52.63	0.03
	Asians			18	1925/2514	1.17 (0.96–1.41)	64.06	0
	T1DM			8	908/1314	1.1 (0.84–1.43)	60.08	0.01
	T2DM			22	2782/3613	1.29 (1–1.66)	85.58	0
					Diseased controls–cases			
NOS3	All	4 b > a	–	17	3887/3196	1.09 (0.96–1.23)	24.04	0.18
	All in HWE			16	3824/3130	1.04 (0.94–1.15)	0	0.73
	Caucasians			7	2147/1659	1.19 (0.96–1.46)	50.17	0.06
	Asians			8	1035/939	1.04 (0.85–1.28)	0	0.51
	T1DM			5	1600/1209	1.24 (0.91–1.68)	65.81	0.02
	T2DM			12	2287/1987	1.04 (0.91–1.18)	0	0.62
					Diseased controls–cases			
NOS3	All	G894T	rs1799983	19	4184/3330	1.19 (0.96–1.48)	79.95	<0.001
	All in HWE			17	3952/3120	1.25 (1.00–1.56)	79.95	<0.001
	Caucasians			4	1778/1105	0.93 (0.81–1.06)	0	0.69
	Asians			11	1586/1548	1.32 (0.90–1.94)	83.08	0
	T1DM			3	1404/870	0.89 (0.76–1.03)	0	1
	T2DM			16	2780/2460	1.28 (0.99–1.70)	80.64	0
					Diseased controls–cases			
MTHFR	All	C677T		26	4246/4380	1.43 (1.16–1.77)	83.91	0
	All in HWE			21	3163/3085	1.28 (1.03–1.59)	78.12	0
	Caucasians			9	1198/1202	1.2 (0.91–1.59)	66.8	0
	Asians			15	2853/2811	1.31 (1.05–1.64)	77.38	<0.001
	T1DM			4	372/667	1.31 (0.88–1.95)	64.75	0.03
	T2DM			22	3874/3713	1.45 (1.14–1.84)	85.58	0
					Three groups			
MTHFR	All	C677T		10	1345/1306/1903	1.44 (1.13–1.83)	84.45	0
	All in HWE			6		1.14 (0.98–1.32)	26.51	0.24
	Caucasians			5	518/595/895	1.09 (0.95–1.25)	64.84	0.37
	Asians			4	734/444/608	1.76 (1.38–2.25)	55.63	0.08
	T1DM			2	153/238/447	1.3 (1.04–1.61)	0	0.7
	T2DM			8	1192/1068/1456	1.47 (1.09–1.98)	87.47	0
					Healthy controls–cases			
MTHFR	All	C677T		13	1507/2503	1.68 (1.15–2.47)	88.17	0
	All in HWE			7		1.21 (0.86–1.7)	69.13	0
	Caucasians			6	585/1167	1.31 (1.02–1.69)	45.04	0.11
	Asians			6	829/936	1.63 (0.97–2.73)	82.88	0
	T1DM			3	174/647	1.71 (1.24–2.37)	83.06	0.34
	T2DM			10	1333/1856	1.67 (1.03–2.71)	90.93	0
					Diseased controls–cases			
AGT	All	M235T	rs699	26	5015/5253	1.21 (1.01–1.45)	82.45	0.00
	All in HWE			19	3181/3655	1.09 (0.92–1.31)	72.76	0.00
	Caucasians			15	2875/2726	1.04 (0.94–1.16)	14.79	0.29
	Asians				1628/2122	1.37 (0.86–2.20)	90.17	<0.001
	T1DM			10	1478/1482	1.12 (0.95–1.32)	34.37	0.13

Table 6. (continued)

	Gene	Variant	RS	Studies (*n*)	Cases/controls (*n*)	RE OR_G (95% CI)	I^2 (%)	P_Q
	T2DM			15	2802/3220	1.31 (0.99–1.72)	86.84	<0.001
					Three groups			
AGT	All	M235T	rs699	9	1156/1350/1243	1.21 (1.02–1.45)	65.91	0.02
	Caucasians			4	827/835/725	1.15 (1.03–1.3)	0	0.94
	Asians			5	329/515/518	1.23 (0.82–1.85)	80.41	0
	T1DM			3	517/482/495	1.19 (1.02–1.38)	0	0.99
	T2DM			6	639/868/748	1.21 (0.9–1.64)	78.5	0
					Healthy cases			
AGT	All	M235T	rs699	12	1399/1759	1.17 (0.87–1.58)	76.47	0
	Caucasians			6	941/1125	1.09 (0.88–1.36)	43.62	0.11
	Asians			6	458/634	1.38 (0.66–2.9)	86.09	0
	T1DM			4	538/695	1.1 (0.78–1.54)	56.02	0.08
	T2DM			8	861/1064	1.26 (0.79–2.01)	82.46	0
					Diseased controls–cases			
AGTR1	All	A1166C	rs5186	24	6000/5020	1.05 (0.93–1.18)	49.13	0.003
	All in HWE			21	5260/4296	0.97 (0.87–1.08)	49.13	0.13
	Caucasians			13	3915/2993	0.94 (0.85–1.03)	10.64	0.34
	Asians			11	2085/2027	1.35 (1.14–1.61)	21.14	0.24
	T1DM			13	3915/2993	0.94 (0.85–1.03)	10.64	0.34
	T2DM			11	2202/2145	1.26 (1.04–1.53)	40.46	0.07
					Diseased controls–cases			
APOE	All	E3 > e2/e4		14	1770/1586	1.08 (0.85–1.37)	57.41	0.00
	All in HWE			13	1341/1170	1.07 (0.81–1.41)	60.62	0.00
	Caucasians			5	589/481	1.15 (0.79–1.67)	48.44	0.1
	Asians			6	1018/897	1.15 (0.89–1.50)	35.62	0.17
	T1DM			9	760/633	1.06 (0.69–1.62)	68.5	0
	T2DM							
					Diseased controls–cases			
TGFB1	All	T869C	rs1800470	11	2408/2452	1.16 (0.94–1.44)	75.49	0
	All in HWE			8	1930/1952	1.08 (0.84–1.38)	77.06	0
	Caucasians			5		1.05 (0.74–1.49)	82.75	0
	Asians			4		1.37 (1.05–1.79)	56	0.08
	T1DM			4		0.86 (0.74–1)	0	0.43
	T2DM			7		1.38 (1.09–1.75)	64.49	0.01

RS: SNP identifier, RE OR_G: random effects odds ratio generalized, I^2: I^2 statistic, PQ: P-value from heterogeneity testing.

The identification of the genetic polymorphisms that contribute to the genetic predisposition of the disease will lead to the discovery of new therapies, but also to more valid and effective means of prevention and prognosis. These discoveries will provide new perspectives in the field of personalized medicine based on the genetic background of each patient and will allow the provision of both preventative and therapeutic interventions at the individual genome level, increasing both the efficacy and the safety of therapies. It is very logical to investigate polymorphisms that have been shown to be statistically significant in association with DN, but agnostic studies that examine the genome as a whole are the most promising.

CONCLUSIONS

Sixty-six genetic polymorphisms have been shown to be associated with DN. These polymorphisms are harboured in 53 gene loc,i while one is not close to any known gene. These results should be interpreted with caution because the true susceptibility loci could be loci that are in linkage disequilibrium with the significant loci. It would be useful in the future to examine haplotypes, study microarray data and perform functional studies for clarification of the role of significant genes to produce newer data, and their combination may lead to the creation of the genetic map of DN.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

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

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