Hum Genet (2010) 127:373–381
DOI 10.1007/s00439-009-0783-x

REVIEW ARTICLE

A meta-analysis of three polymorphisms in the endothelial nitric oxide synthase gene (*NOS3*) and their effect on the risk of diabetic nephropathy

Zhen Zeng · Lina Li · Zhao Zhang · Yang Li · Zhiyun Wei · Ke Huang · Lin He · Yongyong Shi

Received: 17 October 2009 / Accepted: 24 December 2009 / Published online: 5 January 2010

Abstract A number of association studies have investigated the role of the nitric oxide synthase 3 (NOS3) gene in the development of diabetic nephropathy (DN). However, results have been inconclusive, largely because the studies have focused on a variety of different polymorphisms and generate inconsistent results. We performed a meta-analysis of 28 association studies focusing on three polymorphisms in the NOS3 gene (G894T (Glu289Asp), 4b/a, and T-786C) and the risk of DN published before July 2009, covering a total of 10,364 subjects. Although significant heterogeneity was initially found in the analysis of G894T, it did not remain when analysis was done by ethnic subgroups. 894T was negatively associated with DN in Caucasian populations of European origin (OR = 0.896, 0.817–0.983, 95% CI), but was positively associated with DN in East Asian (OR = 2.02, 1.20–3.42, 95% CI) and other populations. Association of the 4b/a variant was observed when studies involving microalbuminuria were excluded (OR = 1.19, 1.02–1.39, 95% CI). The T-786C variant showed an overall weak association (OR = 1.16, 1.01–1.34, 95% CI) with little heterogeneity. In summary, our meta-analysis of the effect of *NOS3* gene polymorphisms on the risk of DN supports the involvement of the *NOS3* gene in the pathogenesis of DN.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-009-0783-x) contains supplementary material, which is available to authorized users.

Z. Zeng · Z. Zhang · Y. Li · Z. Wei · K. Huang · L. He (✉) · Y. Shi (✉)
Bio-X Center, Shanghai Jiao Tong University, Central Little White House, 1954 Huashan Road, 200030 Shanghai, People's Republic of China
e-mail: helin@bio-x.cn

Z. Zeng · Z. Zhang · Y. Li · Z. Wei · K. Huang · L. He · Y. Shi
Affiliated Changning Mental Health Center, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, 200030 Shanghai, People's Republic of China
e-mail: shiyongyong@gmail.com

Z. Zeng · Z. Zhang · Y. Li · Z. Wei · K. Huang · L. He · Y. Shi
Institute for Nutritional Sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 200031 Shanghai, People's Republic of China
e-mail: zzhen@sjtu.edu.cn

L. Li
Department of Biological Science and Biotechnology, Tsinghua University, 10084 Beijing, People's Republic of China

L. He
Institutes of Biomedical Sciences, Fudan University, Ming De Building, 138 Yi Xue Yuan Road, 200032 Shanghai, People's Republic of China

Abbreviations

DN	Diabetic nephropathy
DWN	Diabetes without nephropathy
DM	Diabetes mellitus
NOS3	Nitric oxide synthase 3
ESRD	End-stage renal disease
VNTR	Variable number tandem repeat
LD	Linkage disequilibrium
NADT	No antihypertensive drug treatment
SI	South Indian
NI	North Indian
RFLP	Restriction fragment length polymorphism
TDT	Transmission disequilibrium test

Introduction

Diabetic nephropathy (DN), affecting about one-third of both type 1 and type 2 diabetic patients, is one of the most serious complications of diabetes, and is the leading cause of end-stage renal disease (ESRD) in developed countries (Jacobs and Selwood 1995; Jawa et al. 2004). This life-threatening disorder is characterized by persistent albuminuria, raised arterial blood pressure, a lowered glomerular filtration rate, and high risk of cardiovascular morbidity and mortality (Jawa et al. 2004).

Susceptibility to DN involves many elements, among which the genetic factor has been shown to be significant (Canani et al. 1999; Seaquist et al. 1989). Vascular endothelial dysfunction resulting from impaired nitric oxide synthase 3 (NOS3) in the endothelial cells of blood vessels has been suggested as playing an important role in the pathogenesis of DN (Harrison 1997; Yamada et al. 1995; Palm et al. 2009; Santilli et al. 2004; Nakagawa 2007), and recently, Japanese and American scientists have found that diabetic mice develop advanced DN when *NOS3* gene was knockout (Nakagawa et al. 2007; Mohan et al. 2008; Zhao et al. 2006; Kanetsuna et al. 2007). *NOS3* gene, located in chromosome 7q36, consists of 27 exons and spans 23.5 kb. NOS3 converts L-arginine to L-citrulline and releases nitric oxide (NO) (Moncada and Higgs 1993). NO is a simple molecular structure with important regulatory activities, including vasodilation in glomerular, which is important for the regulation of the glomerular filtration rate (Schmidt and Walter 1994; Klahr 2001).

There have been a number of association studies of polymorphisms in *NOS3* and DN. Three polymorphisms in particular [T-786C (rs2070744), in the promoter region; 4b/a, a 27 bp-repeat VNTR in intron 4 and G894T (rs1799983), which causes a substitution of 298Asp for 298Glu in exon 7 and is also known as Glu298Asp] in *NOS3* have attracted much attention as being potentially relevant and the vast majority of such association studies have investigated at least one of these three.

Although functional experiments support the involvement of NOS3 in the pathogenesis of DN, results of association studies have remained inconsistent. The purpose of our meta-analysis was to evaluate the overall outcomes of all such eligible studies and to identify the source of the discrepancies. Previous studies showed no strong LD between G894T and T-786C in diabetic patients and/or healthy subjects in various populations (Ahluwalia et al. 2008; Ezzidi et al. 2008; Kankova et al. 2007; Liu et al. 2005). The LD between 4b/a and the two other polymorphisms was found to exist in some populations (Ezzidi et al. 2008; Kankova et al. 2007; Mollsten et al. 2006), but not in others (Ahluwalia et al. 2008). Given the absence of strong LD among the three polymorphisms, we analyzed each of them in the meta-analysis.

Materials and methods

Data collection

The literature included in the current analysis was selected using HuGE Navigator (Yu et al. 2008) Literature Finder and by searching the PubMed database with combined keywords "diabetic nephropathy" "diabetes" "endothelial nitric oxide synthase" and the abbreviation of the gene "*eNOS*" "*NOS3*" "*ECNOS*". All references cited by identified eligible studies and previous reviews were scrutinized to find additional work not indexed by PubMed. If necessary, raw data not included in papers were obtained from the author. The analyzed data cover all English and Chinese language publications up to July 2009.

Inclusion criteria

All the studies included satisfied all the following criteria: they (1) were association studies between any of the three polymorphisms in the *NOS3* gene and DN; (2) used diabetic patients without nephropathy as controls; (3) provided genotypes or alleles distribution in both case and control groups; (4) were independent studies and the subject groups investigated did not overlap with each other; (5) were published in peer-reviewed journals and were indexed by PubMed or cited by articles indexed by PubMed. Authors were contacted where clarification was required.

Data extraction

We extracted the following information from each study: first author, journal, publication year, study design, ethnicity of subjects, genotyping method, patient source, definitions and type of diabetes, definitions and number of cases and controls, clinical characteristics (gender, age, and diabetes duration) and matching. The numbers of genotype or allele distribution were extracted or calculated for both cases and controls. Diabetic nephropathy was defined as presenting persistent microalbuminuria, macroalbuminuria, or various stages of chronic renal insufficiency. Units of urinary albumin excretion were defined as in Jawa et al. (2004).

Statistical analyses

The meta-analysis examined the overall and subgroup association of alleles or genotypes and the risk of DN for

each polymorphism. Both dominant and recessive models were applied for genotypic analysis.

Studies were subdivided by ethnicity (Caucasian, including Caucasian of European origin; East Asian, including Chinese, Japanese, Korean and Singaporean; and Mixed, including Asian Indian and Tunisian), genotyping procedures (RFLP versus others), type of diabetes (type 1 or 2), case definitions (including or excluding microalbuminuria), control definitions [diabetes duration of more than 10 years or no antihypertensive drug treatment (NADT)], matched clinical characteristics between cases and controls [gender, age, diabetes duration, age at diabetes onset, body mass index (BMI) and HbA1c], status of Hardy–Weinberg equilibrium (HWE) (yes or no) to find the source of any heterogeneity.

HWE was tested in diabetic patients without nephropathy within each study. Deviation from HWE was tested using the χ^2 test. The effect size was represented by an odds ratio (OR) with 95% confidence interval (CI). Cochran's Q statistic test (DerSimonian and Laird 1986) and I^2 test were used to assess heterogeneity in combined studies or between the subgroups of studies. Publication bias was checked using the Begg test (Begg and Mazumdar 1994) and the Egger test was used for funnel plot asymmetry (Egger et al. 1997a). The random effect model was used to calculate pooled OR (DerSimonian and Laird 1986) with Woolf's 95% CI (Woolf 1955). P values of overall OR were generated using the Z test. Sensitivity analysis was conducted by removing each study and analyzing the others to ensure no single study was totally responsible for overall results. The significance level was set at 0.05, and all P values were two-tailed.

The meta-analysis was performed using Comprehensive Meta Analysis software (Version 2.2.046, BIOSTAT, Englewood, NJ, USA).

Results

Data summary

Different search approaches identified 27 references meeting the criteria. Three of these were discarded after further scrutiny: one because of patient overlap (Komatsu et al. 2002), the second because the ambiguous disease status (Ohtoshi et al. 2002) and the third because of unclear genotype/allele distribution (Nath et al. 2009). A total of 24 references (Ahluwalia et al. 2008; Ezzidi et al. 2008; Kankova et al. 2007; Liu et al. 2005; Mollsten et al. 2006; Cai et al. 1998; Weekers et al. 1999; Neugebauer et al. 2000; Zanchi et al. 2000; Fujita et al. 2000; Degen et al. 2001; Li et al. 2001; Taniwaki et al. 2001; Ukkola et al. 2001; Lin et al. 2002; Taverna et al. 2002; Shimizu et al. 2002; Ksiazek et al. 2003; Rippin et al. 2003; Shin Shin et al. 2004; Dong et al. 2007; Mollsten et al. 2009; Tiwari et al. 2009; Mamoulakis et al. 2009) were eligible, including 30 studies. One TDT study (Zanchi et al. 2000) and one perspective study (Weekers et al. 1999) were excluded on the basis that they used the same samples for the case–control study in each report. Twenty-eight studies eventually met all the criteria for inclusion, and of these 15 focused on the G894T variant, 23 on 4b/a, and 7 on T-786C. Together, these studies included 5,425 cases and 4,939 control subjects. In most of the studies control subjects exhibited HWE (supplementary tables). The analyses revealed no publication bias for any of the variants (supplementary figures).

Association of the G894T (Glu298Asp) variant with DN

The 15 relevant studies included 6,725 individuals (3,585 cases and 3,140 controls) (supplementary table 1). One study (Zanchi et al. 2000) lacked genotype data and was, therefore, excluded from the genotypic analysis. More 894T alleles were found in Caucasian (28.7–36.6%) and Mixed (12.3–34.5%) than in East Asian (5.9–11.7%) control subjects. For the allelic analysis, there was very significant heterogeneity among studies ($P < 0.001$, $I^2 = 63.1\%$), most of which was explained by ethnicity ($P = 0.008$, $I^2 = 79.1\%$) with other factors being much less important (Table 1).

The susceptible allele was found to be different in different populations. Among Caucasians, 894T was negatively associated with DN ($P = 0.020$, OR = 0.896, 0.817–0.983, 95% CI), and there was a complete absence of heterogeneity ($P = 0.789$, $I^2 = 0\%$). In contrast, 894T was positively associated with DN among East Asians ($P = 0.008$, OR = 2.02, 1.20–3.42, 95% CI) and presented no heterogeneity ($P = 0.640$, $I^2 = 0\%$). In the Mixed populations, heterogeneity still existed ($P = 0.001$, $I^2 = 80.6\%$). The sensitivity analysis showed that one study, by Tiwari et al. (SI), was responsible for the heterogeneity in the Mixed populations. When this study was removed, the Mixed populations also showed significant association for 894T ($P = 0.001$, OR = 1.30, 1.12–1.52, 95% CI) without heterogeneity ($P = 0.784$, $I^2 = 0\%$), which was consistent with the results for the East Asian population (Fig. 1). The recessive model results for 894G were consistent with the allelic analysis [Caucasian $P = 0.018$, OR = 0.859, 0.757–0.975, 95% CI; East Asian $P = 0.005$, OR = 2.25, 1.27–3.98, 95% CI; Mixed (with the study of Tiwari et al. (SI) excluded) $P = 0.018$, OR = 1.29, 1.04–1.58, 95% CI]. The dominant model generated a negative result.

Table 1 Heterogeneity between subgroups divided by different characteristics in studies of the G894T polymorphism

Characteristics	Subgroups	Pooled OR	*P* value	I^2
Ethnicity	Caucasian	0.896	0.008	79.121
	East Asian	2.025		
	Mixed	1.074		
DM type	1	0.919	0.319	0.000
	2	1.047		
Genotyping method	RFLP	1.088	0.180	44.423
	Others	0.903		
Microalbuminuria in cases	Y	0.998	0.775	0.000
	N	0.948		
DM >10 years in controls	Y	0.995	0.932	0.000
	N	0.983		
HWE	Y	1.041	0.136	55.074
	N	0.774		
NADT	Y	0.992	0.991	0.000
	N	0.990		
Matched for HbA1c	Y	1.022	0.809	0.000
	N	0.963		
Matched for gender	Y	1.130	0.420	0.000
	N	0.952		
Matched for age	Y	1.054	0.299	7.458
	N	0.911		
Matched for DM duration	Y	1.100	0.573	0.000
	N	0.966		
Matched for age of DM onset	Y	1.087	0.269	18.236
	N	0.929		
Matched for BMI	Y	1.147	0.272	16.954
	N	0.938		

Pooled OR in each subgroup, *P* value of *Q* test between different subgroups, and associated I^2 are shown

Y Yes, *N* no or no data

Association of the 4b/a variant with DN

The 23 studies involving the 4b/a variant included 8,270 individuals (4,465 cases and 3,805 controls) (supplementary table 2). Four studies [Fujita et al., Li et al., Dong et al. (Chinese), Mamoulakis et al.] did not use the recessive model for the 4a allele because the small sample sizes did not provide sufficient data to calculate OR. The frequency of the 4a allele was higher in the control subjects of the Caucasian (13.6–22.4%) than of the East Asian population (6.9–14.3%), and varied widely across the Mixed population (10.7–23.2%). No significant association of the 4a allele ($P = 0.090$, OR = 1.10, 0.96–1.23, 95% CI) and only slight heterogeneity among studies ($P = 0.089$, $I^2 = 29.8\%$) were observed. Ethnicity did not significantly contribute to the heterogeneity. There was no association in the Caucasian, East Asian or Mixed populations. However, we found that when studies involving microalbuminuria in cases were exclude, the association increased to a significant level ($P = 0.028$, OR = 1.19, 1.02–1.39, 95% CI) without heterogeneity ($P = 0.467$, $I^2 = 0\%$) (Fig. 2). The results of the recessive model of genotypic analysis for 4a were consistent with the allelic analysis. We also found significant association in the subgroup without microalbuminuria ($P = 0.018$, OR = 2.14, 1.14–4.00, 95% CI). However, removing each of two studies (Zanchi et al. 2000; Ahluwalia et al. 2008) eliminated the association for both the allelic and recessive models of genotypic analysis (data not shown). The dominant model generated negative outcomes.

Association of the T-786C variant with DN

A total of 3,045 subjects were investigated for T-786C, made up of 1,473 cases and 1,572 controls (supplementary table 3). The -786C allele was more prevalent in controls in Caucasian population (35.2–39.7%) than in the other populations (17.3–25.7%). In the allelic analysis, there was no significant heterogeneity among studies ($P = 0.208$, $I^2 = 28.8\%$). The -786C allele showed an overall association with DN ($P = 0.035$, OR = 1.16, 1.01–1.34, 95% CI) (Fig. 3). However, in the sensitivity analysis, 5 out of the 7 studies produced a *P* value above 0.05. In particular, when the study by Tiwari et al. (SI) was excluded, heterogeneity decreased ($P = 0.336$, $I^2 = 12.4\%$) and the association became stronger ($P = 0.005$, OR = 1.20, 1.06–1.36, 95% CI) (Fig. 4). In the genotypic analysis, the recessive model for the association of the -786C allele showed heterogeneity ($P = 0.034$, $I^2 = 56.0\%$). When the analysis was restricted to three Caucasian studies of European origin, heterogeneity disappeared ($P = 0.307$, $I^2 = 15.3\%$) and the association was significant ($P = 0.006$, OR = 1.61, 1.15–2.25, 95% CI). The dominant model showed negative results.

Haplotype analyses

Four studies investigated the haplotypic association of the three polymorphisms and the risk of DN. They all studied the Mixed population, involving 1,788 subjects (906 cases and 882 controls) (supplementary table 4). Significant heterogeneity was detected ($P = 0.004$, $I^2 = 77.5\%$). When the study of Tiwari et al. (SI) was removed, no heterogeneity existed in the remaining three studies ($P = 0.310$, $I^2 = 14.6\%$), and the most prevalent G–b–T haplotype showed a significant protective role in the risk of DN ($P = 0.002$, OR = 0.765, 0.646–0.905, 95% CI) (Fig. 5).

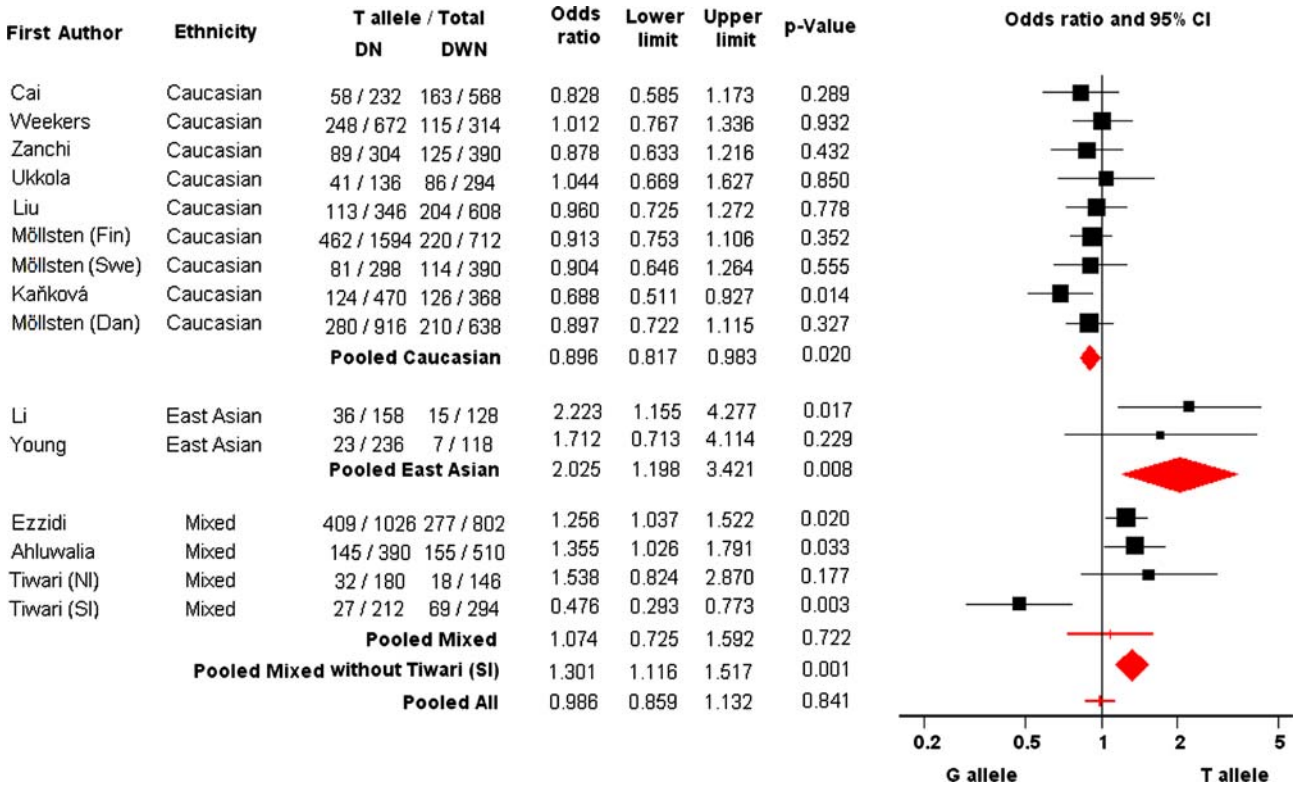

Fig. 1 Meta-analysis of association studies of the G894T polymorphism and DN. Pooled overall OR and ORs within subgroups of different ethnicities are shown. The OR of each study is marked with a *black square*. Pooled ORs are indicated by either *red diamonds* ($P < 0.05$) or *red horizontal lines* ($P > 0.05$)

Discussion

The pathogenesis of the development and progression of DN is far from being clear at present. Variants of the *NOS3* gene may play a role in the NO abnormalities and contribute to the disease (Prabhakar 2004; Freedman et al. 2007). However, results of genetic association studies were confusing because of the difficulty in replicating significant associations. Different characteristics among studies such as ethnicities, DM type, definition of case and control, introduced heterogeneity and made the results of association studies hard to be interpreted. A meta-analysis aiming at finding out the origin of heterogeneity and assessing overall effects of these variants on DN was performed. The meta-analysis covered 10 364 subjects and 28 independent studies on three *NOS3* gene polymorphisms (G894T, 4b/a, and T-786C) reported since 1998, and supported an overall association of the *NOS3* gene with DN.

In the analysis of G894T, ethnicity was responsible for heterogeneity, and the ORs between different DM types, genotyping methods, and case/control definitions were consistent. The 894T allele showed negative association with DN in Caucasian populations but was positively associated with DN in East Asian and Mixed populations. One way to explain this is that G894T may not have a significant effect on the disease, but is in LD with other causative mutations which have not been identified, and the allele associated with the real causative mutations varies across ethnic groups. Another possible explanation is that maybe the variant is influential for the disease, but the genetic backgrounds of the different populations, for instance, different property of other proteins in related pathways, may result in different responses and outcomes. In addition, distinct living environment, habits, and diets, may also mediate the results.

Studies of the 4b/a polymorphism exhibited modest heterogeneity and no overall association. In the subgroup with no microalbuminuria, we observed a significant association without heterogeneity. Given previous findings that patients with microalbuminuria may not necessarily go onto contract progressive nephropathy but may continue to suffer only the milder condition, or even return to normal health (Perkins et al. 2003), the exclusion of such patients probably enhanced the detecting power.

The T-786C polymorphism shows an overall association, but five of the seven relevant studies did not survive the sensitivity analysis test. The results for both the 4b/a and T-786C polymorphisms do not significantly differ

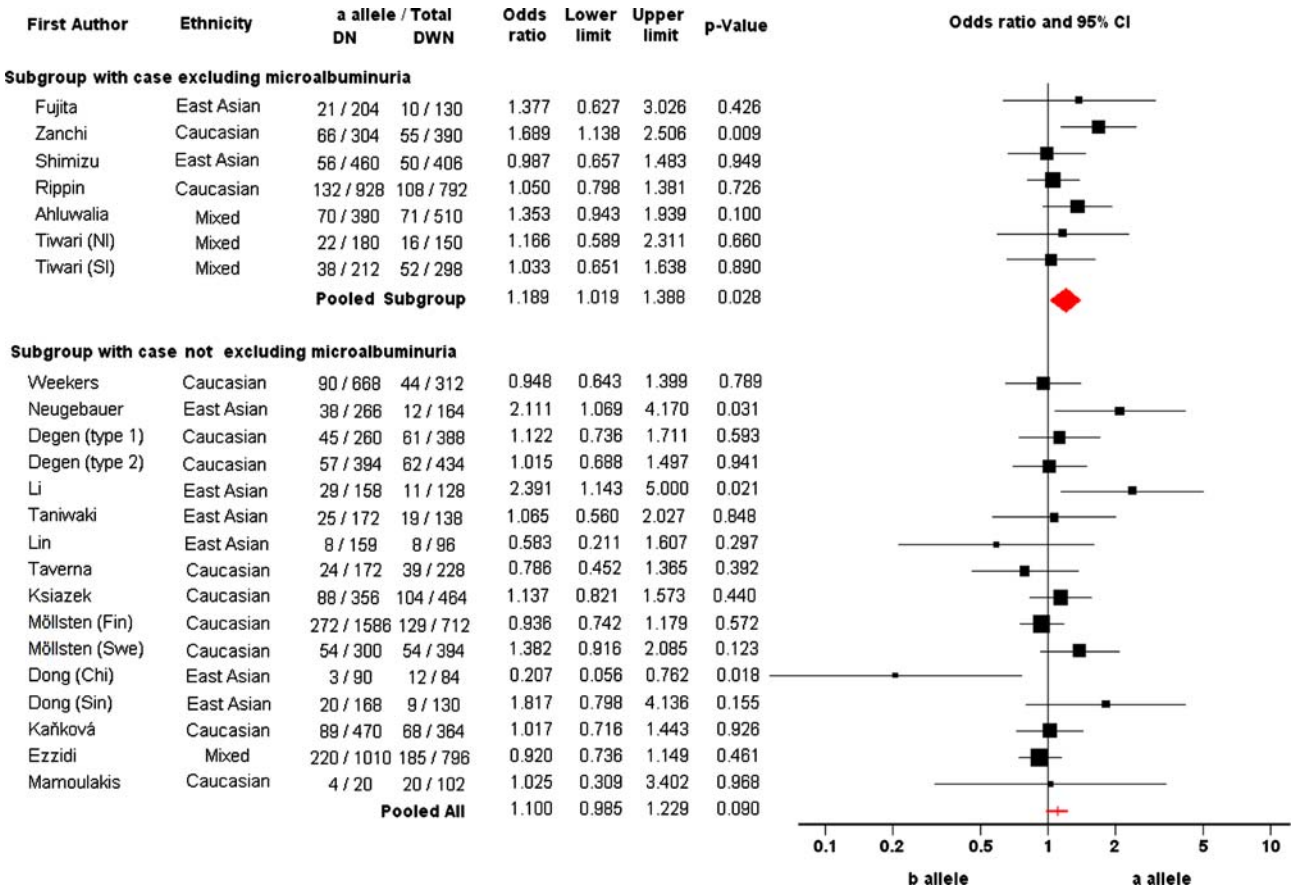

Fig. 2 Meta-analysis of association studies of the 4b/a polymorphism and DN. Pooled OR and the OR within the subgroup with cases excluding microalbuminuria are shown

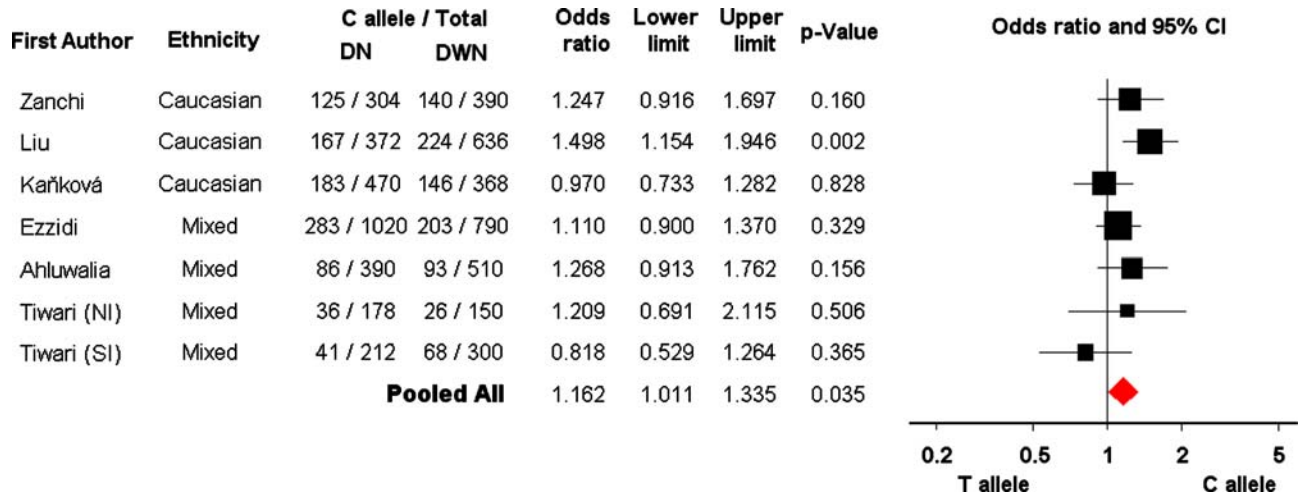

Fig. 3 Meta-analysis of association studies of the T-786C polymorphism and DN. Pooled overall OR is shown

among ethnicities (data not shown). Most of the results generated in allelic analyses of the three polymorphisms were confirmed by the recessive model for the associated alleles. Apart from G894T in the Caucasian populations discussed above, all the minor alleles of the three polymorphisms were associated with DN. The possible protective effect of the combination of the three major alleles is supported by haplotype analyses.

It is worth noting that the study of Tiwari et al. investigating the population of south India (SI) showed results

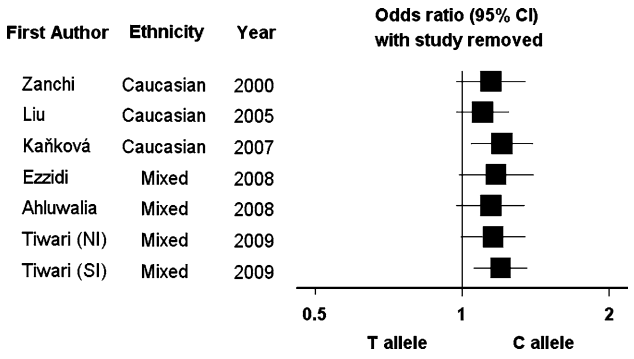

Fig. 4 Sensitivity analysis of the T-786C polymorphism. The pooled OR is presented with each study removed

contrary to other studies in many of the analyses and appeared generally to be the main source of heterogeneity. When this study was excluded from the analyses, associations become stronger with no heterogeneity. The genetic difference of these DM patients from SI may result from a founder effect or from their distinct origin. Omitting this population stratification may decrease the power for detecting genetic risk factors in association studies, as has been demonstrated in studies in the Chinese Han population (Shi et al. 2004). Thus in some of the analyses, results are shown after excluding this study (Figs. 1, 5).

There are a number of studies have previously investigated the functional aspects of the three polymorphisms, but some conclusions are controversial. For G894T, it was reported that protein products with 894T were more susceptible to enzymatic cleavage (Tesauro et al. 2000). Tesauro et al. (2000) and Fairchild et al. (2001), however, failed to find difference in NO generation between the two alleles in transiently transfected COS7 cells. Noiri et al. (2002) observed a significant lower NO production in stably transfected Chinese hamster ovary 894T cells. While Ritt et al. (2008) found G894T had no impact on basal NO activity in the renal circulation of patients with or without DM, Ahluwalia et al. (2008) found serum NO levels were significantly lower in diabetic 894T allele carriers. For 4b/a, Wang et al. (2002) reported that this 27 bp repeat in intron 4 might have a *cis*-regulating effect on the NOS3 promoter. Tsukada et al. (1998) detected significant decreased plasma NO metabolites levels in 4a allele carriers in healthy subjects. But the finding was not repeated by Ahluwalia et al. (2008) in diabetic patients. The T-786C variant has been suspected to influence the promoter activity in *NOS3*. Nakayama et al. (1999) found that the -786C allele was associated with a significant reduction in *NOS3* gene transcription rate using luciferase reporter gene assay. Miyamoto et al. (2000) identified a protein (replication protein A1, RPA1) that might function as a repressor of transcriptional activity of the *NOS3* gene with the -786C allele. They also found significant diminished serum NO products levels among the -786C allele carriers. The same conclusion was reached by Ahluwalia et al. (2008) among diabetic patients.

Although these findings regarding G894T, 4b/a, and T-786C had some implications, none of them has been convincingly proved as determinants responsible for the development of DN, their associations are still very likely to be due to in LD with other causative mutations in the *NOS3* gene.

Taking certain limitations and bias into account, the results of our meta-analysis should be treated with caution. Overall, we did not detect substantial publication bias (supplementary figures), but underlying bias may be within subgroups. And since we only included Chinese and English studies, it may introduce language bias (Egger et al. 1997a, b). In addition, most of included studies are retrospective. A contradictory fact exists in the study design. To ensure that patients in control group have less chance to develop DN later, individuals with long DM duration should be recruited as controls. However, in DM duration matched case group, the lower survival of DN patients carrying the "risk" allele may cause bias that will reduce

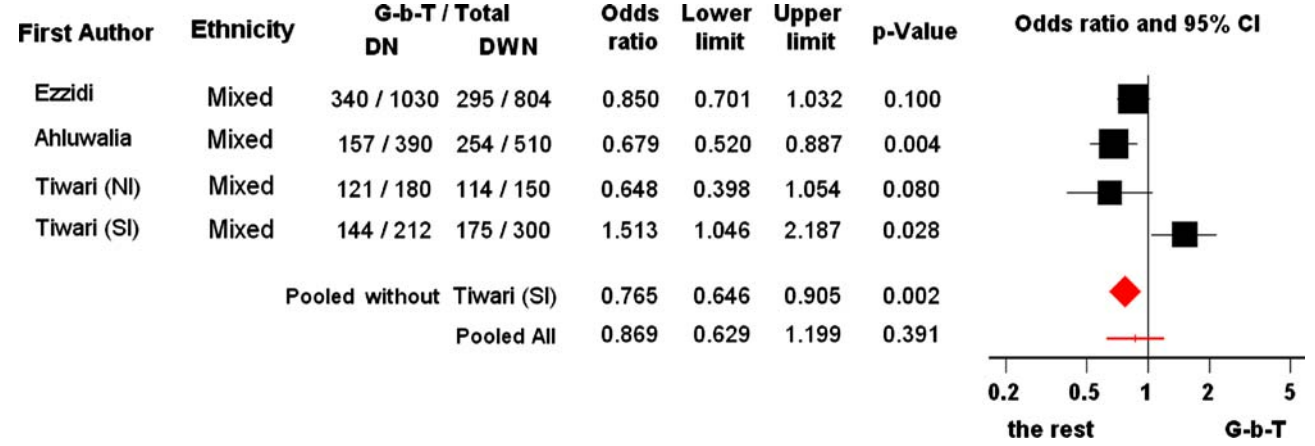

Fig. 5 Meta-analysis of the G–b–T haplotype and the risk of DN

power. This contradiction can only be solved by perspective study design. What's more, although we extracted 13 characteristics from eligible studies and estimated their contributions to the heterogeneity separately, the combined effect of different characteristics can hardly be estimated. Also, we cannot take some environmental factors such as living habits and diets into account. Finally, in some subgroups, especially in East Asians of G894T, the sample size is small. Results of these subgroups should be treated very carefully.

To conclude, the current meta-analysis supports the effects of three polymorphisms in the *NOS3* gene on the risk of DN. We also found that genetic heterogeneity exists among ethnicities. In order to further demonstrate the involvement of *NOS3* gene in the progress of DN, the screening of new functional variants is necessary. And it is warranted to undertake TDT studies in different populations with larger sample size and more matched clinical characteristics to avoid environmental and other confounding factors. However, to identify the exact role of *NOS3* gene polymorphisms in the pathogenesis of DN and to clarify the function of NOS3 in the disease mechanisms of DN, there is an important role for studies such as functional analyses and animal disease modeling.

Acknowledgments This work was supported by Grants (2006AA02A407, 2007CB947300, 07DZ22917, 2010CB529600, and 09DJ1400601), the Shanghai Changning Health Bureau Program (2008406002), the Shanghai Municipal Health Bureau Program (2008095), and the Shanghai Leading Academic Discipline Project (B205). Special thanks are due to Dr Tiwari, Prof. T.B.K., Dr Liu, Dr Kaňková, and Prof. Marre for their help in providing the raw data and material from their earlier work.

References

Ahluwalia TS, Ahuja M, Rai TS et al (2008) Endothelial nitric oxide synthase gene haplotypes and diabetic nephropathy among Asian Indians. Mol Cell Biochem 314(1–2):9–17

Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50(4):1088–1101

Cai H, Wang X, Colagiuri S, Wilcken DE (1998) A common Glu298 → Asp (894G → T) mutation at exon 7 of the endothelial nitric oxide synthase gene and vascular complications in type 2 diabetes. Diabetes Care 21(12):2195–2196

Canani LH, Gerchman F, Gross JL (1999) Familial clustering of diabetic nephropathy in Brazilian type 2 diabetic patients. Diabetes 48(4):909–913

Degen B, Schmidt S, Ritz E (2001) A polymorphism in the gene for the endothelial nitric oxide synthase and diabetic nephropathy. Nephrol Dial Transplant 16(1):185

DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3):177–188

Dong JJ, Zhao JJ, Lee KO, Lir MC, Chan SW, Liao L (2007) Distribution of polymorphism in endothelial nitric oxide synthase gene in Singapore Chinese and its association with diabetic nephropathy. Zhonghua Yi Xue Za Zhi 87(48):3415–3417

Egger M, Davey Smith G, Schneider M, Minder C (1997a) Bias in meta-analysis detected by a simple, graphical test. BMJ 315(7109):629–634

Egger M, Zellweger-Zahner T, Schneider M, Junker C, Lengeler C, Antes G (1997b) Language bias in randomised controlled trials published in English and German. Lancet 350(9074):326–329

Ezzidi I, Mtiraoui N, Mohamed MB, Mahjoub T, Kacem M, Almawi WY (2008) Association of endothelial nitric oxide synthase Glu298Asp, 4b/a, and -786T>C gene variants with diabetic nephropathy. J Diabetes Complications 22(5):331–338

Fairchild TA, Fulton D, Fontana JT, Gratton JP, McCabe TJ, Sessa WC (2001) Acidic hydrolysis as a mechanism for the cleavage of the Glu(298) → Asp variant of human endothelial nitric-oxide synthase. J Biol Chem 276(28):26674–26679

Freedman BI, Bostrom M, Daeihagh P, Bowden DW (2007) Genetic factors in diabetic nephropathy. Clin J Am Soc Nephrol 2(6):1306–1316

Fujita H, Narita T, Meguro H et al (2000) Lack of association between an ecNOS gene polymorphism and diabetic nephropathy in type 2 diabetic patients with proliferative diabetic retinopathy. Horm Metab Res 32(2):80–83

Harrison DG (1997) Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest 100(9):2153–2157

Jacobs C, Selwood NH (1995) Renal replacement therapy for end-stage renal failure in France: current status and evolutive trends over the last decade. Am J Kidney Dis 25(1):188–195

Jawa A, Kcomt J, Fonseca VA (2004) Diabetic nephropathy and retinopathy. Med Clin North Am 88(4):1001–1036

Kanetsuna Y, Takahashi K, Nagata M et al (2007) Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. Am J Pathol 170(5):1473–1484

Kankova K, Stejskalova A, Pacal L et al (2007) Genetic risk factors for diabetic nephropathy on chromosomes 6p and 7q identified by the set-association approach. Diabetologia 50(5):990–999

Klahr S (2001) The role of nitric oxide in hypertension and renal disease progression. Nephrol Dial Transplant 16(Suppl 1):60–62

Komatsu M, Kawagishi T, Emoto M et al (2002) ecNOS gene polymorphism is associated with endothelium-dependent vasodilation in Type 2 diabetes. Am J Physiol Heart Circ Physiol 283(2):H557–H561

Ksiazek P, Wojewoda P, Muc K, Buraczynska M (2003) Endothelial nitric oxide synthase gene intron 4 polymorphism in type 2 diabetes mellitus. Mol Diagn 7(2):119–123

Li C, Dong Y, Lu W (2001) The association between polymorphism of endothelial nitric oxide synthase gene and diabetic nephropathy. Zhonghua Nei Ke Za Zhi 40(11):729–732

Lin S, Qu H, Qiu M (2002) Allele A in intron 4 of ecNOS gene will not increase the risk of diabetic nephropathy in type 2 diabetes of Chinese population. Nephron 91(4):768

Liu Y, Burdon KP, Langefeld CD et al (2005) T-786C polymorphism of the endothelial nitric oxide synthase gene is associated with albuminuria in the diabetes heart study. J Am Soc Nephrol 16(4):1085–1090

Mamoulakis D, Bitsori M, Galanakis E, Vazgiourakis V, Panierakis C, Goulielmos GN (2009) Intron 4 polymorphism of the endothelial nitric oxide synthase eNOS gene and early microangiopathy in type 1 diabetes. Int J Immunogenet 36(3):153–157

Miyamoto Y, Saito Y, Nakayama M et al (2000) Replication protein A1 reduces transcription of the endothelial nitric oxide synthase gene containing a −786T → C mutation associated with coronary spastic angina. Hum Mol Genet 9(18):2629–2637

Mohan S, Reddick RL, Musi N et al (2008) Diabetic eNOS knockout mice develop distinct macro- and microvascular complications. Lab Invest 88(5):515–528

Mollsten A, Wessman M, Svensson M et al (2006) Glu298Asp and NOS4ab polymorphisms in diabetic nephropathy. Ann Med 38(7):522–528

Mollsten A, Lajer M, Jorsal A, Tarnow L (2009) The endothelial nitric oxide synthase gene and risk of diabetic nephropathy and development of cardiovascular disease in type 1 diabetes. Mol Genet Metab 97(1):80–84

Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med 329(27):2002–2012

Nakagawa T (2007) Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 292(6):F1665–F1672

Nakagawa T, Sato W, Glushakova O et al (2007) Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol 18(2):539–550

Nakayama M, Yasue H, Yoshimura M et al (1999) T-786 → C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 99(22):2864–2870

Nath SD, He X, Voruganti VS et al (2009) The 27-bp repeat polymorphism in intron 4 (27 bp-VNTR) of endothelial nitric oxide synthase (eNOS) gene is associated with albumin to creatinine ratio in Mexican Americans. Mol Cell Biochem 331(1–2):201–205

Neugebauer S, Baba T, Watanabe T (2000) Association of the nitric oxide synthase gene polymorphism with an increased risk for progression to diabetic nephropathy in type 2 diabetes. Diabetes 49(3):500–503

Noiri E, Satoh H, Taguchi J et al (2002) Association of eNOS Glu298Asp polymorphism with end-stage renal disease. Hypertension 40(4):535–540

Ohtoshi K, Yamasaki Y, Gorogawa S et al (2002) Association of (-)786T-C mutation of endothelial nitric oxide synthase gene with insulin resistance. Diabetologia 45(11):1594–1601

Palm F, Teerlink T, Hansell P (2009) Nitric oxide and kidney oxygenation. Curr Opin Nephrol Hypertens 18(1):68–73

Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS (2003) Regression of microalbuminuria in type 1 diabetes. N Engl J Med 348(23):2285–2293

Prabhakar SS (2004) Role of nitric oxide in diabetic nephropathy. Semin Nephrol 24(4):333–344

Rippin JD, Patel A, Belyaev ND, Gill GV, Barnett AH, Bain SC (2003) Nitric oxide synthase gene polymorphisms and diabetic nephropathy. Diabetologia 46(3):426–428

Ritt M, Ott C, Delles C, Schneider MP, Schmieder RE (2008) Impact of the endothelial nitric oxide synthase gene G894T polymorphism on renal endothelial function in patients with type 2 diabetes. Pharmacogenet Genomics 18(8):699–707

Santilli F, Cipollone F, Mezzetti A, Chiarelli F (2004) The role of nitric oxide in the development of diabetic angiopathy. Horm Metab Res 36(5):319–335

Schmidt HH, Walter U (1994) NO at work. Cell 78(6):919–925

Seaquist ER, Goetz FC, Rich S, Barbosa J (1989) Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 320(18):1161–1165

Shi Y, Zhao X, Yu L et al (2004) Genetic structure adds power to detect schizophrenia susceptibility at SLIT3 in the Chinese Han population. Genome Res 14(7):1345–1349

Shimizu T, Onuma T, Kawamori R, Makita Y, Tomino Y (2002) Endothelial nitric oxide synthase gene and the development of diabetic nephropathy. Diabetes Res Clin Pract 58(3):179–185

Shin Shin Y, Baek SH, Chang KY et al (2004) Relations between eNOS Glu298Asp polymorphism and progression of diabetic nephropathy. Diabetes Res Clin Pract 65(3):257–265

Taniwaki H, Ishimura E, Matsumoto N, Emoto M, Inaba M, Nishizawa Y (2001) Relations between ACE gene and ecNOS gene polymorphisms and resistive index in type 2 diabetic patients with nephropathy. Diabetes Care 24(9):1653–1660

Taverna MJ, Sola A, Guyot-Argenton C et al (2002) eNOS4 polymorphism of the endothelial nitric oxide synthase predicts risk for severe diabetic retinopathy. Diabet Med 19(3):240–245

Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J (2000) Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci USA 97(6):2832–2835

Tiwari AK, Prasad P, KT B et al (2009) Oxidative stress pathway genes and chronic renal insufficiency in Asian Indians with Type 2 diabetes. J Diabetes Complications 23(2):102–111

Tsukada T, Yokoyama K, Arai T et al (1998) Evidence of association of the ecNOS gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 245(1):190–193

Ukkola O, Erkkila PH, Savolainen MJ, Kesaniemi YA (2001) Lack of association between polymorphisms of catalase, copper-zinc superoxide dismutase (SOD), extracellular SOD and endothelial nitric oxide synthase genes and macroangiopathy in patients with type 2 diabetes mellitus. J Intern Med 249(5):451–459

Wang J, Dudley D, Wang XL (2002) Haplotype-specific effects on endothelial NO synthase promoter efficiency: modifiable by cigarette smoking. Arterioscler Thromb Vasc Biol 22(5):e1–e4

Weekers L, Hadjadj S, Belloum R, Gallois Y, Bouhanick B, Marre M (1999) Lack of contribution of two endothelial nitric oxide synthase (eNOS) gene polymorphisms to diabetic nephropathy in type 1 diabetes. Diabetologia 42:A266 (Abstract)

Woolf B (1955) On estimating the relation between blood group and disease. Ann Hum Genet 19:251–253

Yamada K, Nakano H, Nakayama M et al (1995) Endothelium-dependent relaxation in peripheral vasculature and kidney of non-insulin-dependent diabetes mellitus. J Diabetes Complications 9(4):203–207

Yu W, Gwinn M, Clyne M, Yesupriya A, Khoury MJ (2008) A navigator for human genome epidemiology. Nat Genet 40(2):124–125

Zanchi A, Moczulski DK, Hanna LS, Wantman M, Warram JH, Krolewski AS (2000) Risk of advanced diabetic nephropathy in type 1 diabetes is associated with endothelial nitric oxide synthase gene polymorphism. Kidney Int 57(2):405–413

Zhao HJ, Wang S, Cheng H et al (2006) Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. J Am Soc Nephrol 17(10):2664–2669