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Abstract

Background: High plasma adiponectin levels are associated with diabetic nephropathy (DN). T-cadherin gene (*CDH13*) variants have been shown to be associated with adiponectin levels. We investigated associations between allelic variations of *CDH13* and DN in subjects with type 1 diabetes.

Methods: Two *CDH13* polymorphisms were analysed in 1,297 Caucasian subjects with type 1 diabetes from the SURGENE (n=340, 10-year follow-up), GENESIS France-Belgium (n=501, 5-year follow-up for n= 462) and GENEDIAB (n=456, 9-year follow-up for n=283) cohorts. Adiponectin levels were measured in plasma samples from GENESIS and GENEDIAB cohorts.

Results: Pooled analysis of GENEDIAB and GENESIS studies showed that baseline plasma adiponectin levels were higher in subjects with established/advanced DN at inclusion ($p<0.0001$) and in subjects who developed end-stage renal disease (ESRD) at follow-up ($p<0.0001$). The minor allele of rs3865188 was associated with lower adiponectin levels ($p=0.006$). Rs11646213 (OR 1.47; 95%CI 1.18-1.85; $p=0.0009$) and rs3865188 (OR 0.71, 95%CI 0.57-0.90, $p=0.004$) were associated with high baseline prevalence of established/advanced DN. These polymorphisms were also associated with the risk of ESRD ($0.006<p<0.03$). The association between rs11646213 (but not rs3865188) and renal function remained significant after adjustment for plasma adiponectin. In SURGENE, rs11646213 (HR 1.69, 95% CI 1.01-2.71, $p=0.04$) and rs3865188 (HR 0.74, 95% CI 0.55-0.99, $p=0.04$) were associated with an excess risk of renal events (defined as progression to more severe DN stages).

Conclusions: Plasma adiponectin levels are associated with the prevalence of DN and the incidence of ESRD in patients with type 1 diabetes. *CDH13* polymorphisms were also associated with the prevalence and incidence of DN, and with the incidence of ESRD in these

patients. The association between *CDH13* and diabetic nephropathy may be due to pleiotropic effects, both dependent and independent of plasma adiponectin levels.

Keywords

Adiponectin; diabetic nephropathy; genetic polymorphisms; T-cadherin; type 1 diabetes;

Abbreviations

CI, confidence interval; DN, diabetic nephropathy; ESRD, end-stage renal disease; GWAS, genome-wide association studies; HR, hazard ratio; OR, odds ratios; SNP, single-nucleotide polymorphisms; UAE, urinary albumin excretion

Short summary

T-cadherin gene (*CDH13*) variants have been shown to be associated with adiponectin levels. We investigated associations between two *CDH13* polymorphisms and diabetic nephropathy in subjects with type 1 diabetes. *CDH13* polymorphisms and plasma adiponectin levels were associated with the prevalence of DN and the incidence of ESRD in patients with type 1 diabetes. The association between *CDH13* and diabetic nephropathy may be due to pleiotropic effects, both dependent and independent of plasma adiponectin levels.

Introduction

Diabetic Nephropathy (DN) is a major microvascular complication in type 1 diabetic patients leading to end-stage renal disease (ESRD) [1]. One-half of patients with type 1 diabetes currently develop nephropathy, which increases the risk of mortality [2]. Various genetic factors modulate susceptibility to renal disease [3,4].

Adiponectin is an adipokine mainly secreted by adipocytes, with anti-inflammatory, anti-atherogenic and insulin-sensitizing properties [5]. Nevertheless, plasma adiponectin levels are increased in patients with type 1 diabetes [6–8]. Baseline plasma adiponectin was higher in patients with type 1 diabetes who developed nephropathy in the SURGENE prospective study [9]. In patients with type 1 diabetes and DN, adiponectin was predictive of end-stage renal disease incidence [10]. However, adiponectin levels may decrease in early stages of nephropathy and increase in more advanced stages [11]. In patients with type 2 diabetes, increased plasma adiponectin levels are generally correlated with impaired renal function [12-14]. In subjects with type 2 diabetes as well as type 1 diabetes, polymorphisms of the gene coding for adiponectin, *ADIPOQ*, associated with high adiponectin levels, were also associated with an increased risk of nephropathy [14,15].

T-cadherin is a cell adhesion protein anchored to cell membrane by a glycosylphosphatidylinositol anchor. It is deprived of its transmembrane segment, but still takes part in cellular signal cascades contributing to cell-cell adhesion, cell polarity and morphogenesis actions [16,17]. T-cadherin is highly expressed in renal arteries [18] and is expressed abundantly in injured vascular endothelial cells [16]. T-cadherin co-locates with adiponectin at the site of vascular injury [19,20]. The coexistence of both molecules at the vascular endothelium is necessary for their action on revascularization [21]. Their colocalization on cardiomyocytes also appears to be critical for the cardioprotective action of adiponectin [20]. In genome-wide association studies (GWAS), plasma adiponectin levels

have been associated not only with *ADIPOQ* polymorphisms, but also with T-cadherin gene (*CDH13*) polymorphisms [22,23]. T-cadherin selectively binds high-molecular-weight and medium-molecular-weight adiponectin, but it remains unclear whether T-cadherin is a receptor for adiponectin [16,19]. T-cadherin might act as a co-receptor for adiponectin receptors Adipo R1 and/or Adipo R2, or an as yet unknown receptor [20].

Given the relationship between adiponectin and DN and between T-cadherin and adiponectin, the present study was designed to assess a possible association between *CDH13* polymorphisms and DN in subjects with type 1 diabetes, related to plasma adiponectin concentrations.

Subjects and Methods

Participants

We studied three cohorts designed to evaluate the genetic components of diabetic nephropathy. GENESIS France-Belgium and "Génétique de la Néphropathie Diabétique" (GENEDIAB) were cross-sectional, multicentre, binational (Belgium and France) studies. GENESIS was family-based, including 662 probands with type 1 diabetes and a diagnosis of diabetic retinopathy [25]. We studied 501 probands for whom DNA samples were available, including 279 individuals (55.7%) with a diagnosis of incipient, established or advanced diabetic nephropathy. GENEDIAB included 456 patients with type 1 diabetes and a diagnosis of severe diabetic retinopathy [26]. Incipient, established or advanced diabetic nephropathy was present in 310 individuals (69.8%). In a prospective observational study, a subset of GENEDIAB (n=229) and GENESIS (n=456) participants were followed for 9 ± 3 and 6 ± 3 years, respectively. The incidence during follow-up of new cases of ESRD, defined as need for renal replacement therapy, was 11.4% for GENEDIAB and 5.5% for GENESIS.

The “Survival Genetic Nephropathy” (SURGENE) was a prospective study conducted in 340 subjects with type 1 diabetes, recruited consecutively over a 3-year period in the outpatient clinic of Angers University Hospital, France [27]. At baseline, 60 individuals (17.6%) had a diagnosis of incipient, established or advanced diabetic nephropathy (see below). Mean duration of follow-up was 10±3 years. New cases of incipient nephropathy (persistent microalbuminuria in consecutive biannual assessments) during follow-up were observed in 76 of the 280 subjects with normal baseline urinary albumin excretion (27.1%). A renal event, defined as new cases of incipient nephropathy or progression to a more severe stage of nephropathy, was observed in 98 (28.8%) participants during follow-up. At the end of the study, 136 individuals (40%) had a diagnosis of incipient, established or advanced diabetic nephropathy. All participants from the three cohorts provided their written informed consent and study protocols were approved by the Angers University Hospital ethics committee, France.

Diabetic nephropathy and retinopathy stages

Diabetic nephropathy was categorized as: no nephropathy, defined as urinary albumin excretion (UAE) <30 mg/24 h or <20 µg/min or <20 mg/l and plasma creatinine <150 µmol/l on at least two of three consecutive assessments and in the absence of antihypertensive treatment; incipient nephropathy, defined as persistent microalbuminuria (UAE = 30–300 mg/24 h or 20–200 µg/min or 20–200 mg/l) and plasma creatinine <150 µmol/l on at least two of three consecutive assessments; established nephropathy, defined as past or present macroalbuminuria (UAE>300 mg/24 h or >200 µg/min or >200 mg/l) and plasma creatinine <150 µmol/l; and advanced nephropathy, defined as past or present macroalbuminuria and plasma creatinine >150 µmol/l or renal replacement therapy. In GENESIS-GENEDIAB, UAE was expressed in mg/l in 72% of patients, mg/24 h in 27% of patients, and µg/min in 1% of patients. In the single-centre SURGENE study, UAE was expressed in mg/l for all patients.

Estimation of the glomerular filtration rate (eGFR) was computed with the Modification of Diet in Renal Disease (MDRD) formula [26]. Retinopathy was staged according to Kohner's classification as non-proliferative, pre-proliferative or proliferative retinopathy.

Adiponectin assays

Total adiponectin was assayed in fasting plasma EDTA samples from 928 subjects of the GENESIS and GENEDIAB cohorts. Samples were collected at baseline and kept frozen at -80°C. An ELISA kit from Alpco®, Eurobio, Courtaboeuf, France was used. According to the manufacturer, intra- and inter-assay coefficients of variation for adiponectin assay were less than 6.0%.

Genotyping

Two single-nucleotide polymorphisms (SNP) in the promoter region of *CDH13* (chromosome 16q23.3) were selected based on previous studies showing an association with adiponectin levels and/or other metabolic traits [22,23,29]. Genotypes were determined by competitive allele-specific PCR genotyping system assays (KASP, LGC Genomics, Hoddesdon, UK). Genotyping success rate was higher than 97%. Genotypes were in Hardy-Weinberg equilibrium (Pearson's Chi-square test with 1 degree of freedom $p>0.05$).

Statistical analysis

Results are expressed as mean ± SD except when otherwise specified. For all analyses of quantitative parameters, data were log-transformed when the normality of the distribution was rejected by the Shapiro-Wilk W test. Differences between groups were assessed by Pearson's Chi-square test, Fisher's exact test, ANOVA and ANCOVA. Multiple linear regression analysis was used to test the associations between plasma adiponectin and renal parameters (eGFR, UAE). Cox proportional hazards survival analysis was used to examine the effect of explanatory variables on time-related survival rates in prospective analyses.

Logistic regression analysis was used for cross-sectional analyses of nephropathy. Hazard ratios (HR) or odds ratios (OR), respectively, with their 95% confidence intervals (CI) were computed in these analyses for the minor alleles. Competing risk regression analysis (Fine and Gray model) was performed to estimate subhazard ratios of ESRD by considering death as a competing risk.

To increase statistical power, GENEDIAB and GENESIS cohorts were pooled for genetic analyses, with appropriate covariate adjustments to take into account cohort differences. In all cohorts, individuals with established or advanced nephropathy were also pooled for genetic analyses. Statistical analyses were adjusted for age, sex, duration of diabetes, BMI and, when applicable, cohort membership. To take into account differences in diabetic retinopathy status in the various cohorts resulting from different inclusion criteria, all comparisons of diabetic nephropathy by genotype included adjustment for retinopathy stages. Correction for multiple comparisons due to multiple SNP testing took into account the effective number of independent tests (Meff) based on the degree of linkage disequilibrium between SNPs. A moderate linkage disequilibrium was observed between the 2 SNPS (D'=-0.90 and R^2= 0.49, in GENESIS-GENEDIAB). A p value $\leq$0.033 was therefore considered to be significant for genotype-related comparisons in the discovery cohort (GENESIS/GENEDIAB), unless otherwise specified. The tests concerning polymorphisms correspond to the best fitting models of inheritance according to descriptive statistics (additive, dominant or recessive). Statistical analyses were performed with JMP software (SAS Institute Inc., Cary, NC), except for competing risk analysis (Stata Software, StataCorp LP, College Station, TX) and haplotype analyses (THESIAS software, INSERM U525, Paris, France) [30].

Results

GENESIS and GENEDIAB cohorts: renal status and adiponectin levels

The baseline prevalences of incipient and established or advanced diabetic nephropathy in the pooled GENESIS and GENEDIAB cohorts were 22.3% and 40%, respectively. Characteristics of participants with or without nephropathy have been previously published [3].

The cumulative incidence of ESRD during follow-up was 7.4% (N=51) in the pooled cohorts. Baseline clinical characteristics of participants according to ESRD status at follow-up have been previously published [4]. Briefly, compared to patients without ESRD, patients with *de novo* ESRD, were younger, had a shorter duration of diabetes, higher levels of HbA1c, blood pressure and UAE, decreased renal function, and were more often taking antihypertensive treatment (supplementary Table 1).

Median baseline values (interquartile range 0.25-0.75) of plasma adiponectin levels for absent, incipient and established/advanced nephropathy were 7.1 (4.8-10.5) µg/mL, 7.5 (5.0-11.0) µg/mL and 11.3 (7.5-16.9) µg/mL ($p=0.02$ and $p<0.0001$ for incipient vs. absent and established/advanced vs. absent, respectively) (ANCOVA on Log-transformed values, adjusted for sex, age, duration of diabetes, BMI, retinopathy and cohort membership). In multiple regression analysis, plasma adiponectin was correlated with both UAE and eGFR ($p<10^{-6}$ for both). The correlation with UAE remained significant ($p<10^{-6}$) after further adjustment for eGFR, and conversely, the correlation with eGFR remained significant ($p<10^{-6}$) after adjustment for UAE.

Plasma adiponectin levels were also higher in patients who developed ESRD during follow-up compared to those who did not: 16.2 (10.4-22.9) vs 7.5 (4.9-11.0) µg/mL, respectively, ($p<0.0001$). This association remained significant after adjustment for baseline nephropathy status.

GENESIS and GENEDIAB cohorts: association of CDH13 polymorphisms with adiponectin levels and renal traits

The distribution of plasma adiponectin levels by genotype for each polymorphism are illustrated in Table 1. In the pooled GENESIS and GENEDIAB cohorts, plasma adiponectin levels were significantly associated with rs3865188, as subjects carrying a minor allele A had lower plasma adiponectin levels (p=0.006). No significant association was observed between plasma adiponectin levels and rs11646213.

Genotype frequencies by baseline diabetic nephropathy stages are shown in Table 2. We observed a positive association between the minor allele A of rs11646213 (OR 1.47, 95% CI 1.18-1.85, p=0.0009) and an inverse association between the minor allele A of rs3865188 (OR 0.71, 95% CI 0.57-0.90, p=0.004) with the prevalence of established/advanced nephropathy at baseline. These results are in keeping with genotype-related prevalences of established/advanced nephropathy: 31.9% (TT), 39.1% (TA) and 45% (AA) for rs11646213, and 40.3% (TT), 37.6% (TA) and 28.2% (AA) for rs3865188. Subjects carrying the rs11646213 A allele also had higher diastolic blood pressure (p=0.01) and higher levels of urinary albumin excretion (p=0.01). Subjects with the AA genotype of rs3865188 had lower levels of UAE (p=0.04). No significant association was found between *CDH13* SNPs and eGFR as a quantitative phenotype (data not shown).

CDH13 polymorphisms were associated with ESRD during follow-up (Table 3). The cumulative incidence of ESRD during follow-up according to genotype was 7.4% (TT), 5.3% (TA) and 12.8% (AA) for rs11646213 and 11.5% (TT), 4.6% (TA) and 7.7% (AA) for rs3865188. Cox proportional hazards survival analyses confirmed these associations: HR 1.96, 95% CI 1.06-3.51, p=0.03 for the A-allele of rs11646213, and HR 0.47, 95% CI 0.27-0.84, p=0.009 for the A-allele of rs3865188 (Table 3, supplementary Figure 1). Competing

risk regression analyses were performed to estimate subhazard ratios (SHR) for *CDH13* polymorphisms as a risk for ESRD, by considering all-cause mortality as a competing risk. SHR and HR from the Cox model were similar, indicating that death was not a competing risk in the association between *CDH13* polymorphisms and ESRD (data not shown).

The associations between rs3865188 and the prevalence of established/advanced nephropathy and the incidence of ESRD were no longer significant after adjustment for plasma adiponectin levels.

The haplotype containing both alleles at risk for rs11646213 and rs3865188, AT, was significantly associated with the prevalence of established/advanced nephropathy (OR 1.41, 95% CI 1.09-1.83, p=0.009) and the incidence of ESRD (HR 1.73, 95% CI 1.10-2.73, p=0.02) compared to the opposite haplotype, TA. These associations persisted after further adjustment for adiponectin levels for both established/advanced nephropathy (OR 1.43, 95% CI 1.09-1.88, p=0.01) and ESRD (HR 1.51, 95% CI 1.04-2.19, p=0.03).

SURGENE cohort: association between CDH13 SNPs and renal phenotypes

The cumulative incidence of renal events during follow-up was 28.8% (n=98). Baseline characteristics of the participants according to the incidence of renal events during follow-up have been previously published [3] (supplementary table 1).

Genotype frequencies by renal event status during follow-up are shown in Table 4. The incidence of renal events during follow-up according to genotype was 30.6% (TT), 27.2% (TA) and 44.4% (AA) for rs11646213, suggesting a positive association between the minor A-allele and renal events, and 38.5% (TT), 29.4% (TA) and 21.7% (AA) for rs3865188, suggesting an inverse association with the minor A-allele in an additive model. Cox proportional hazards survival analyses confirmed the positive association between A-allele of rs11646213 (HR 1.69, 95% CI 1.01-2.71, p=0.04) and the inverse association between the A-allele of rs3865188 (HR 0.74, 95% CI 0.55-0.99, p=0.04) and renal events

(Table 4, supplementary Figure 2). The haplotype containing both alleles at risk for rs11646213 and rs3865188, AT, was significantly associated with the incidence of renal events (HR 1.45, 95% CI 1.06-1.99, p=0.02) compared to the opposite haplotype, TA.

Discussion

The present study demonstrated associations between plasma adiponectin levels and the prevalence and incidence of diabetic nephropathy and ESRD in cohorts of subjects with type 1 diabetes. *CDH13* polymorphisms were associated with the prevalence of established/advanced DN, the incidence of ESRD, and urinary excretion of albumin and diastolic blood pressure in the GENESIS and GENEDIAB cohorts. The association between renal disease and rs3865188 was no longer significant after adjustment for plasma adiponectin, while the associations with rs11646213 and the haplotype including both alleles at risk remained significant after adjustment, indicating that the association between *CDH13* and diabetic nephropathy may be due to pleiotropic effects, both dependent and independent of plasma adiponectin levels. These genetic associations were validated in SURGENE, another cohort of subjects with type 1 diabetes, in which we observed an association between these polymorphisms and progression of DN during follow-up.

Our results on plasma adiponectin levels are consistent with previous studies showing associations of high plasma adiponectin levels with diabetic nephropathy in people with type 2 diabetes [12-14] or type 1 diabetes [9, 10]. In particular, we have previously shown that high plasma adiponectin levels precede nephropathy in type 1 diabetes in the SURGENE cohort [9]. Nevertheless, the mechanism underlying the relationship between plasma adiponectin and nephropathy remains unclear. Renal failure *per se* may lead to stimulation of adiponectin production as a physiological counter-regulatory response to restrict endothelial damage [31]. It may also decrease adiponectin clearance [32], and the kidney may develop

secondary resistance to adiponectin [33]. However, the prospective design of the present study and several previous studies, tends to indicate a causal relationship. Nevertheless, higher plasma adiponectin levels were associated with lower urinary albumin excretion rates at an early stage of the disease, which would argue against this hypothesis [34]. However, Menzaghi *et al.* [35] showed a positive association of HMW adiponectin with albuminuria in untreated non-diabetic subjects. Sharma *et al.* have shown that albuminuria was negatively correlated with plasma adiponectin in obese African-American subjects without diabetes or kidney disease [36]. In a murine model, adiponectin also reduced albuminuria and improved podocyte function by reducing oxidative stress [36]. On the other hand, at an advanced stage of the disease, higher plasma adiponectin levels were associated with more severe chronic kidney disease [37] and a higher risk of progression of nephropathy [13,14].

In GWAS, *CDH13* allele polymorphisms have been associated with variations in plasma adiponectin levels [23,38]. In the present study, the minor allele of rs3865188 was also associated with lower plasma adiponectin levels [38]. However, the association between *CDH13* rs3865188 and renal traits was no longer significant after adjustment for plasma adiponectin levels. It is therefore tempting to consider that the relationship between *CDH13* polymorphisms and diabetic nephropathy is mediated by plasma adiponectin levels. Our results are in favour of a deleterious role of plasma adiponectin in diabetic nephropathy. We have previously shown that two *ADIPOQ* variants, associated with high plasma adiponectin levels, were also predictive of the development of DN in type 2 diabetic subjects [14]. The same *ADIPOQ* SNPs have also been associated with nephropathy in subjects with type 1 diabetes [15]. In individuals with normal kidney function, one of these *ADIPOQ* SNPs was also positively associated with both plasma adiponectin and albuminuria [35].

T-cadherin-deficient mice have very high plasma adiponectin levels, but develop cardiomyopathy due to the absence of tissue binding of adiponectin [20]. T-cadherin has been

shown to be primarily responsible for binding and localizing adiponectin to skeletal muscle, vascular endothelium and myocardium [21]. About 75% of the body's total adiponectin appears to be bound to tissue via a T-cadherin-dependent mechanism [39]. It can therefore be hypothesized that the *CDH13* alleles associated with lower plasma adiponectin levels might also be associated, via an unknown mechanism, with higher T–cadherin expression and increased tissue binding of adiponectin. Such a relationship has already been described [40]. In this case, a decrease in plasma adiponectin levels would correspond to more intense binding and consequently higher adiponectin uptake. The variants studied are located in the promoter region and first intron of *CDH13* and could be associated with gene expression. If adiponectin had a deleterious effect on the kidney, the *CDH13* rs3865188 A allele associated with lower plasma adiponectin levels, but putative higher adiponectin binding, should be associated with a higher risk of nephropathy. However, we observed the opposite association in the present study, suggesting that adiponectin could be harmful to the kidneys, but without binding to T-cadherin. Another hypothesis is that the relationship between *CDH13* variants and plasma adiponectin is modified by type 1 diabetes. In another study on the D.E.S.I.R. cohort derived from the general population, rs3865188 A was associated with high plasma adiponectin levels and rs11646213 A was associated with low plasma adiponectin levels [41]. The rs11646213 A variant was not associated with plasma adiponectin in the present study.

Until the causal mechanisms of these associations have been studied, we cannot rule out the hypothesis that T-cadherin might also act on the kidney independently of adiponectin. Our results support the hypothesis of both plasma adiponectin dependent and independent effects. T-cadherin plays a functional role in differentiation of podocytes and formation of the glomerular capillary network [42]. *CDH13* genetic variants might therefore impact on these functions. At baseline, the rs11646213 and rs3865188 variants were associated with the prevalence of DN and urinary albumin excretion. The genotype associated with more severe

nephropathy was also associated with higher levels of urinary albumin excretion, which could suggest a role of T-cadherin in kidney disease via urinary albumin excretion.

No association of CDH13 with kidney function and chronic kidney disease in type 1 diabetic patients was observed in GWAS [43,44]. Nevertheless, rs11646213 was associated with blood pressure traits in a GWAS conducted in two European populations [22],-which is in line with our results, since the minor allele A of rs11646213 was associated with higher baseline diastolic blood pressure in a pooled GENESIS and GENEDIAB cross-sectional analysis. Moreover, in the Framingham Heart Study, another *CDH13* SNP was associated with long-range blood pressure [45]. Consistent with these data, the 16q23.1 genomic region has also been linked to blood pressure in linkage scans [46,47]. T-cadherin regulates vascular wall remodelling and angiogenesis [18]. Renal artery impairment, leading to microalbuminuria, is a major cause of secondary hypertension and might be linked to *CDH13* genetic variants.

It has been claimed that increased albuminuria and decreased GFR have different pathogenic backgrounds. Diabetic nephropathy might be the combined result of many disease states that may be under distinct genetic control [44]. In the present study, we showed a very strong association between plasma adiponectin and both UAE and eGFR, but *CDH13* SNPs were only associated with UAE. Although their putative effect might be partly due to adiponectin, this supports the hypothesis of different genetic backgrounds for the various components of diabetic nephropathy.

To our knowledge, this is the first report of an association between T-cadherin gene polymorphisms and renal disease in subjects with type 1 diabetes. The other strengths of our study are the detailed phenotype assessment of renal function during follow-up in the three cohorts, the number of subjects included in the study, as well as validation of our findings in an independent cohort. However, our study has several limitations. Although total plasma

adiponectin levels were assayed, high-molecular-weight (HMW) adiponectin and other isoforms were not measured. HMW adiponectin is the more active form of the protein, but its relationship with kidney disease remains unclear. It has sometimes been associated with DN [13], but, in our previous study in subjects with type 2 diabetes, only low and medium molecular weight adiponectin were associated with the onset of new renal events [14]. Plasma T-cadherin levels were not assayed in the present study [48]. Plasma T-cadherin assays could allow more accurate evaluation of the relationship between these proteins and the development of DN in subjects with type 1 diabetes. Another limitation of our study is the relatively small sample size of our cohorts and the limited number of outcomes during follow-up. The statistical power might have been insufficient to detect effects of small magnitude, especially in the SURGENE cohort.

In conclusion, genetic variants of *CDH13* were associated with the prevalence, incidence and progression of DN in subjects with type 1 diabetes, as well as the incidence of ESRD. Our findings suggest that T-cadherin may play an important role in the pathogenesis of diabetic nephropathy in these patients. The association between *CDH13* and diabetic nephropathy may be due to pleiotropic effects, both dependent and independent of plasma adiponectin levels.

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Conflicts of interest: none

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Table 1. Baseline plasma adiponectin levels according to genotype

		Plasma adiponectin[a] (µg/mL)	p
rs11646213	TT	8.4 (5.4-12.5)	0.98
	TA	8.3 (5.3-12.6)	
	AA	8.3 (5.3-11.8)	
rs3865188	TT	8.6 (5.7-12.4)	0.02
	TA	8.2 (5.2-12.8)	
	AA	7.8 (4.9-12.0)	

MAF: minor allele frequency. ANCOVA on Log transformed values adjusted for sex, age, duration of diabetes, BMI, retinopathy and cohort membership
[a] values are expressed as median (interquartile range)

Table 2. GENESIS-GENEDIAB pooled study: genotype frequencies according to the baseline prevalence of diabetic nephropathy

		No nephropathy (n=356)	Incipient nephropathy (n=211)	Established and advanced nephropathy (n=378)	OR (95% CI) for incipient nephropathy	p	OR (95% CI) for established and advanced nephropathy	p
rs11646213	TT	0.34	0.38	0.27	1.11 (0.86-1.43)	0.43	1.47 (1.18-1.85)	0.0009
	TA	0.52	0.38	0.50				
	AA	0.14	0.24	0.23				
	MAF	0.40	0.43	0.48				
rs3865188	TT	0.28	0.38	0.37	0.80 (0.63-1.03)	0.08	0.71 (0.57-0.90)	0.004
	TA	0.51	0.40	0.49				
	AA	0.21	0.22	0.14				
	MAF	0.47	0.42	0.39				

Logistic regression adjusted for sex, age, duration of diabetes, BMI, retinopathy stages and cohort membership.
Odds ratios and p-values for the minor allele in additive models
MAF: minor allele frequency.

Table 3. GENESIS-GENEDIAB pooled study: genotype frequencies according to the incidence of End-Stage Renal Disease during follow-up

		End-Stage Renal Disease			
	Genotype	Yes	No	HR (95% CI)[a]	p[a]
rs11646213	N	49	601	1.96 (1.06 – 3.51)	0.03
	TT	0.33	0.33		
	TA	0.33	0.48		
	AA	0.34	0.19		
	MAF	0.51	0.43		
rs3865188	N	49	607	0.47 (0.27 – 0.84)	0.01
	TT	0.51	0.32		
	TA	0.29	0.48		
	AA	0.20	0.20		
	MAF	0.35	0.44		

Cox proportional hazards survival analysis model, adjusted for sex, age, duration of diabetes BMI, retinopathy stages and cohort membership.

[a] Hazard ratio for the minor allele in a recessive model for rs11646213 and a dominant model for rs3865188

MAF: minor allele frequency.

Table 4. SURGENE study: genotype frequencies according to the incidence of renal events during follow-up

Polymorphism		**Renal events during follow-up**			
SNP		Yes (n=98)	No (n=242)	HR (95% CI)[a]	p
rs11646213	TT	0.34	0.34	1.69 (1.01-2.71)	0.04
	TA	0.43	0.52		
	AA	0.23	0.14		
	MAF	0.45	0.40		
rs3865188	TT	0.38	0.27	0.74 (0.55-0.99)	0.04
	TA	0.46	0.49		
	AA	0.16	0.24		
	MAF	0.39	0.49		

Cox proportional hazards survival analysis model, adjusted for sex, age, duration of diabetes, BMI and retinopathy stages. MAF: minor allele frequency.

[a] Hazard ratio for the minor allele in a recessive model for rs11646213 and in a dominant model for rs3865188