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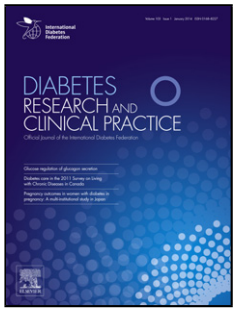

Title: Adiponectin gene (ADIPOQ) polymorphisms correlate with the progression of nephro<!–<query id="Q1">Please provide a reduced form of the main title that doesn't exceed 80 characters.</query>–><!–<RunningTitle>Adiponectin gene (*ADIPOQ*) polymorphisms correlate with the progression of nephro</RunningTitle>–>pathy in Taiwanese male patients with type 2 diabetes

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Adiponectin gene (*ADIPOQ*) polymorphisms correlate with the progression of nephropathy in Taiwanese male patients with type 2 diabetes

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Running title: *ADIPOQ* polymorphisms predict diabetic nephropathy

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Abstract

Aims: Polymorphisms of the *ADIPOQ* gene were associated with diabetic nephropathy (DN) in case-control studies predominantly among European populations. Gender may modify the *ADIPOQ* associated risk for DN. We investigated the association of 18 *ADIPOQ* polymorphisms with DN in a prospective Taiwanese cohort of type 2 diabetes (T2D) and explored whether gender plays a role in this genetic association.

Methods: Selected single nucleotide polymorphisms (SNPs) of *ADIPOQ* were genotyped in 566 T2D patients with normoalbuminuria at baseline. DN was defined based on urinary albumin-to-creatinine ratio (ACR). The Cox proportional hazard model was used to explore the association of individual SNP to DN events under different genetic models over a 6-year follow-up period. Analyses were further stratified by gender.

Results: In male patients, the adjusted hazard ratios under the recessive models were 1.81 for rs2241766 TT (vs. GT+GG, 95% CI=1.10-2.96, p=0.019) and 1.89 for rs1063537 CC (vs. CT+TT, 95% CI=1.15-3.11, p=0.013). In the Kaplan-Meier survival curve, males carrying rs2241766 TT (vs. GT+GG, p=0.050) and rs1063537 CC (vs. CT+TT, p=0.037) recessive homozygotes also had a reduced nephropathy-free survival rate. SNPs rs2241767 and rs2082940, both in strong correlation with tag SNP rs1063537 ($r^2 \geq 0.96$), were also associated with DN progression in males. In females, *ADIPOQ* polymorphisms were not associated with the progression of DN.

Conclusions: *ADIPOQ* genetic polymorphisms rs2241766 (+45T>G), rs1063537, rs2241767 and rs2082940 were correlated with the progression of DN in Taiwanese male patients with T2D. The role of gender in this *ADIPOQ* genetic association needs to be further investigated in other

populations.

Keywords: *ADIPOQ*; diabetic nephropathy; gender-specific; polymorphism

Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) [1], and affects more patients with type 2 diabetes (T2D) than those with type 1 diabetes (T1D) [2]. A global study has reported that the overall prevalence of albuminuria in T2D to be about 50% [3]. Asian and Hispanic patients have the highest prevalence of albuminuria (55%), while Caucasians have the lowest (40.6%) [3], indicating possible ethnic differences. The pathogenesis of DN is multifactorial, and thought to come about as a result of environmental and genetic factors [4]. Genetic factors play an important role in the observed ethnic disparity in the development of DN [4], and gender may predispose patients to the development of kidney disease, as both animal and human studies have reported a higher incidence and a faster progression rate of the disease among males [5-7].

The adiponectin gene (*ADIPOQ*), located on human chromosome 3q27, has been mapped as a genetic susceptibility locus for obesity, insulin resistance, T2D, and cardiovascular disease (CVD) in different populations [8,9]. Adiponectin, encoded by *ADIPOQ*, is lower in patients with T2D and CVD due to its insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties [10]. However, an increase in circulating adiponectin is found in patients with kidney diseases [11-13]. Polymorphisms in the two small regions of *ADIPOQ*, one in the promoter and another in the boundary of exon 2-intron 2, are the most commonly discussed variants. To date, a small number of case-control studies have reported an association between *ADIPOQ* polymorphisms and DN among T1D and T2D patients [14-19]. However, these studies were carried out predominantly among Europeans with only one being conducted in a Chinese population [16]. Zhang et al have recently reported an association between promoter polymorphism

rs17300539 (-11391A/G) and DN in European female subjects with T1D, though the biological basis of gender predisposition is not very well understood [14,15].

In this prospective study, we first genotyped a larger number of 18 *ADIPOQ* polymorphisms, and then examined their genetic effects on the progression of DN in Taiwanese T2D patients. We further explored whether gender plays a role in this genetic association.

Materials and Methods

Study subjects

The study subjects were diagnosed T2D patients enrolled in a study project involving diabetes management through an integrated delivery system (DMIDS) (ClinicalTrials.gov NCT00288678). Details of the study design and inclusion/exclusion criteria have been reported elsewhere [20]. Briefly, 1,209 T2D patients were recruited at baseline (August 2003 to December 2005) and followed until the end of 2009. We excluded 367 patients who did not have available specimens to perform *ADIPOQ* genotyping and 276 who had urinary albumin-to-creatinine ratio (ACR) ≥30 mg/g in at least one of the first two urine tests, leaving us with 566 subjects for genetic association analysis. This study was approved by the Ethics Committee of the National Health Research Institutes and Kaohsiung Medical University Hospital, Taiwan. Written informed consent was obtained from each subject.

Clinical assessments and primary end point

Anthropometric data (including height, weight, systolic and diastolic blood pressure), fasting venous blood (overnight≥8h), and morning spot urine were collected every 6 months. Glucose, triglycerides, cholesterol, LDL-C, HDL-C and creatinine were measured by an automatic analyser

(Hitachi 7060; Hitachi High Technologies, Tokyo, Japan). HbA1c was measured by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA) and urinary albumin by immunoturbidimetry (Hitachi 7060; Hitachi High Technologies, Tokyo, Japan). All samples were kept in 2-8°C, delivered to a central laboratory, and measured within 8 hours. Adiponectin was determined by a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN, USA), using plasma samples cross-sectionally collected in 2008. Duplicate measurements were performed and coefficient of variation less than 10% were calculated for quality control.

The end point of this study was progression to DN, which has been classically defined by the presence of microalbuminuria. In our cohort, microalbuminuria was defined as having urinary ACR ≥30 mg/g in two consecutive urine tests [20].

SNP selection and genotyping

We performed 18 single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene. Nine of these SNPs were tag SNPs with minor allele frequencies (MAF) >5% in the HapMap Han Chinese population. Tag SNPs, including rs16861194 (-11426A>G), rs266729 (-11377C>G), rs182052, rs822394, rs12495941, rs7627128, rs1501299 (+276G>T), rs3774261 and rs1063537, were selected. Two common SNPs, rs17300539 (-11391G>A) and rs2241766 (+45T>G, also known as +94T>G), were included in the selection based on previous review and meta-analysis studies [21,22]. The remaining seven SNPs (rs4632532, rs16861205, rs822396, rs2241767, rs3821799, rs6773957 and rs2082940) were genotyped in an attempt to duplicate/reproduce our findings, since they were highly correlated with one of the tag SNPs ($r^2 \geq 0.8$).

Genomic DNA was isolated from human leukocytes using standard methods and stored at -20℃ until genotyping. The GenomeLabTM

SNPstream ® genotyping platform (Beckman Coulter Inc., Fullerton, CA, USA) and its accompanying SNPstream software suite were used to perform multiplex polymerase chain reaction (12-plex PCR) and SNP genotyping. The PCR primers were designed to amplify DNA, and probes were designed to identify the SNP. The SNPstream genotyping assay was performed according to methods previously described [23]. All SNPs were accurately genotyped with a call rate >95%.

Statistical analysis

Chi-squared or Student's t-tests were used to compare baseline characteristics between nephropathy and non-nephropathy groups whenever appropriate. One-sample Kolmogorov-Smirnov test was used to examine the normality of the continuous variables, and a log transformation to normalize the skewed distribution. Pairwise |D'| and r^2 values between SNPs were computed using the Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). Frequencies of allele and genotype between the DN and non-DN groups were examined by Chi-squared or Fisher's exact test. Each SNP in the non-DN control group was tested for deviation from Hardy–Weinberg equilibrium using X^2 goodness-of-fit test. To examine the gender-specific effect on genetic risk for DN, we further stratified by gender.

Kaplan-Meier estimates, log-rank tests, and univariate Cox proportional hazard models were used to explore the association of individual SNP with time to new DN events under different specifications of the genetic model, including allelic, additive, dominant and recessive models. Patient data were censored at the end of the study or the time of death or loss to follow-up. A hazard ratio (HR) in Cox proportional hazard models refers to the hazard of a (set of) risk genotype over the other non-risk genotypes of a SNP on developing nephropathy. Multivariate Cox proportional hazard models were further adjusted for diabetes duration, education (≤6, >6 years), blood pressure (≥140/90, <140/90mmHg), HbA1C, triglyceride, and use of ACEI or ARB (yes, no) at baseline to determine the independent genetic effects. Analyses of female patient data were additionally adjusted for

menopause status (pre-, post-menopause). We evaluated proportional hazard assumptions using log-log survival plots or time interactions for all covariates, with no violations noted.

Posterior study power was estimated for each SNP associated with progression of DN separated by gender, using the genetic power calculator [24]. We also included 267 patients with urinary ACR≥30 mg/g at baseline to perform a case-control analysis in an attempt to investigate this genetic association in a larger sample size (total n=842, males=396, Supplemental Table 1 and 2). The independent association between *ADIPOQ* polymorphisms and DN was examined using multivariate logistic regression models.

ANCOVA analyses were carried out to examine the adjusted mean plasma concentrations of adiponectin among genotypes stratified by disease status. All statistical operations were performed using the SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). A two-sided P value <0.05 was considered significant.

Results

Baseline characteristics of study subjects

The baseline characteristics of the 566 selected T2D patients are shown in Table 1. Of these subjects, 263 (46.5%) were male, overall mean age was 55.2±8.4 years, and average diabetes duration was 4.6±5.5 years. Over a follow-up period of 6 years, 144 (25.4%) patients who had normoalbuminuria at baseline progressed to DN. Patients who progressed to DN were less educated and had a longer diabetes duration, higher concentrations of HbA1C and ACR at baseline ($p<0.05$). Additionally, they also tended to have higher blood pressure and increased ACEI or ARB medication use, although the differences only reached marginal significance. In female patients, we found that females with DN had a lower creatinine level than females without DN (55.3 vs.59.6 μmol/L, p=0.024). Generally, patients with increases in urinary albumin had increased serum

creatinine levels. It is possible that variations in muscle mass between the two groups may contribute to lower creatinine level observed in DN subjects. Assessment of body composition was unavailable for this cohort study. In the case-control design (n=842), 420 patients were defined as cases (DN group) in the end of 2009, who had a high prevalence (about 50%) of DN.

LD analysis

The G to A polymorphism at rs17300539 (-11391G>A) was not found in our population. We, therefore, performed linkage disequilibrium (LD) analyses and association analyses based on the remaining 17 SNPs. As shown in Fig. 1, tag SNP rs1063537 was in complete LD with rs2241767 (r^2=1) and in high LD with rs2082940 (r^2=0.96). SNP rs2241766 was in strong LD with rs1063537 (r^2=0.88), as were rs2241767 and rs2082940. Therefore, only rs2241766 and the 9 tag SNPs were presented without losing the power of using all SNPs. The allele and genotype frequencies in this cohort were all comparable to those in the HapMap Han Chinese population. In the non-DN group, the genotype distributions were consistent with Hardy-Weinberg equilibrium (p>0.05), except for rs266729, rs822394 and rs822396.

Distribution and association of ADIPOQ polymorphisms with DN risk

Overall, the distributions of *ADIPOQ* alleles and genotypes were not statistically different between nephropathy and non-nephropathy groups (Table 2). When analysed separately by gender, frequency of rs1063537 C allele was significantly higher in patients with DN progression compared to those without that progression in males (75.0% vs. 65.5%, p=0.040), but not in females (p=0.366) (Table 3). The HR for DN was 1.50 (95% CI: 1.03-2.21, p=0.037) for C allele in males. We also found that distribution of rs2241766 T allele was higher in male subjects with DN progression (74.3% vs. 65.5%), however only reaching marginal significance (p=0.058). Additionally, frequencies of rs2241766 TT and rs1063537 CC genotype

were slightly higher in male patients with DN progression (chi-squared p=0.099-0.133), and those differences became significant in a larger sample size of case-control analysis (chi-squared p=0.002-0.006) (Table 3 and Supplemental Table 1). The odds ratio (OR) for DN was 2.61 (95% CI: 1.14-5.95, p=0.023) for rs2241766 TT genotype and 3.10 (95% CI=1.33-7.20, p=0.009) for rs1063537 CC genotype (Supplemental Table 1).

Survival analyses under different genetic models

The associations between *ADIPOQ* SNPs and the risk of DN progression under different genetic models are shown in Table 4. In males, based on comparison of genotype effects, the recessive model was the most appropriate genetic model in this study, in which the adjusted HRs were 1.81 (95% CI=1.10-2.96, p=0.019) for rs2241766 TT (vs. GT+GG) and 1.89 (95% CI=1.15-3.11, p=0.013) for rs1063537 CC (vs. CT+TT). In the additive model, rs2241766 and rs1063537 were also significantly associated with DN, with respective adjusted HRs of 1.66 (p=0.021) and 1.73 (p=0.013), suggesting a dose-additive manner. In the Kaplan-Meier survival curve estimate under the recessive model, we also found that male patients carrying rs2241766 TT (vs. GT+GG, p=0.050) and rs1063537 CC (vs. CT+TT, p=0.037) homozygotes had a reduced nephropathy-free survival rate compared to the reference group (Fig. 2). Additionally, two SNPs rs2241767 and rs2082940, both highly correlated with tag SNP rs1063537 ($r^2 \geq 0.96$), were also associated with the development of DN under the allelic, additive and recessive models among males (data not shown). In females, *ADIPOQ* SNPs were not associated with DN progression. We did not find an association between menopause status and DN in either uni- or multi-variate model, although sex hormone might be one plausible explanation of the gender difference in the development of DN.

Study power of a genetic association largely relies on the frequencies of risk allele and the prevalence of disease of interest. Due to a high frequency of risk allele at approximately 0.7 and a high prevalence of DN in T2D of at least 30%, our sample size of male patients was calculated to have a power of 85.9-89.7% to detect an HR of 1.81-1.89 for DN under the recessive model at α level of 0.05. Additionally, the association of

rs2241766 and rs1063537 with the risk of DN found in the prospective design was replicable in case-control design using multivariate logistic regression models (Supplemental Table 2).

Levels of plasma adiponectin

The mean adiponectin plasma concentration in our study was 6.9±6.0 ng/ml. Patients with nephropathy had slightly higher concentrations than those without nephropathy (7.5±7.0 vs. 6.7±5.6 ng/ml, p=0.318), but the differences did not reach statistical significance (Table 1). The adiponectin concentrations in females were significantly higher than in males in either nephropathy (8.7 vs. 6.3 ng/ml, p<0.001) or non-nephropathy groups (8.1 vs. 5.0 ng/ml, p=0.005). We also found that post-menopausal women had a higher level of circulating adiponectin than pre-menopausal women (9.27 vs. 6.31 ng/ml, p<0.001, data not shown).

Discussion

In this cohort of Taiwanese T2D patients, we found the rs2241766 (+45T>G) TT, rs1063537 CC, rs2241767 AA and rs2082940 CC recessive homozygotes of the *ADIPOQ* to be associated with a greater risk for progression to DN in male T2D patients but not in females over a 6 year follow-up period. Males carrying these recessive homozygotes also had a reduced nephropathy-free survival rate. These four SNPs in our sample were highly correlated (r^2>0.8), as they have also been reported among Europeans [25].

Only two prospective studies to date have found an association between +45T>G and DN risk in T2D. The +45G allele was associated with an increased risk of incident renal event in the French population [26], and the GG genotype of +45T>G was associated with DN in Korean patients [27]. The findings of both studies were inconsistent with ours as we found that +45T allele or TT genotype carriers had a greater risk for DN

progression. This discrepancy may be partially explained by differences in ethnic backgrounds. The mechanisms underlying the association of +45T>G polymorphisms with DN progression in the Taiwanese or Chinese population remain to be determined. Other case-control studies carried out among European T1D patients showed that SNPs in the promoter region, not in the exon region (+45T>G), may confer susceptibility to the risk of DN [14,15,17]. Among studies of Han-Chinese populations, only one Taiwanese case-control study found that a potential interaction among *ADIPOQ* (-11377C>G), *GHSR* and *TCF7L2* might contribute to the risk of DN in T2D, though no single *ADIPOQ* genetic effect was found [16]. Most of previous studies [14-19] were performed using case-control designs and only analyzed a few common SNPs. The present study represents one of the few prospective investigations in the Chinese population examining the association between *ADIPOQ* genetic variations and the progression of DN with a wide selection of 18 *ADIPOQ* SNPs.

In the Chinese population, there has been extensive investigation of the association of +45T>G polymorphism with metabolic disease and CVD. A few Taiwanese studies have associated the mutant G allele at +45T>G with improved insulin sensitivity [28] and reduced risks of obesity [29] and coronary artery disease [30]. These findings suggest that +45G allele may confer a protective effect against metabolic disease and CVD in the Taiwanese population. On the other hand, in other regions of China, meta-analysis studies have reported that the G allele does not consistently show protective effects on metabolic syndrome [31], T2D [22], or CVD [32]. These contrasting results in different regions of Chinese might be explained by regional variations and different study methods including diagnostic criteria, sample size, and study design.

Gender is another important factor to consider in DN development since males have a more rapid progression to this complex disease than females [7]. The ratio is about 1.3:1 [33]. In the present study, the *ADIPOQ* associated risk for DN was only found in males, but not in females. However, Zhang et al [14,15] reported that European female T1D patients, but not males, had *ADIPOQ* genetic risk for DN. One Japanese study also reported gender differences in the association of *ADIPOQ* but with T2D in male subjects [34]. Considering gender factor into genetic research seems

to be more complicated, and the effects are not universal in different populations. Besides genetic factor, other factors such as differences in diet, nephron number or renal mass and the direct/potential effects of sex hormone may also explain the gender predisposition in the development of kidney disease [7]. Estrogens may confer protective effect on kidney disease as a result of its potent antioxidant actions in the mesangial microenvironment [6]. To date, many studies are still accumulating evidence to address the questions of gender and ethnic disparities and to elucidate the biological mechanisms underlying gene-gender interactions in such a complex disease.

Increased concentrations of circulating adiponectin have been found in patients with ESRD and DN [11-13]. In the present study, we did not observed a statistically significant difference in adiponectin concentrations between DN and non-DN groups. This could be explained by the finding that most of our T2D patients were in early stage of kidney disease (over 90% of patients with a glomerular filtration rate $\geq$60ml/min/1.73m^2 at baseline). Due to the beneficial effects of adiponectin, an increase in its concentrations among patients with kidney disease may result from the physiological counter regulatory response which is up-regulated to reduce endothelial damage and renal insufficiency, and which could contribute to the development of secondary resistance to adiponectin [11,13]. In addition, we found an increased circulating adiponectin level in post-menopausal females. This could also be explained by adiponectin resistance or reduction in adiponectin clearance associated with renal function decline after menopause [35].

The +45T>G polymorphism, a synonymous mutation (Gly→Gly) in the exon region, may affect expression levels of adiponectin by altering pre-mRNA splicing [36]. One possible explanation for the absence of an association of +45T>G or other SNPs with circulating adiponectin in the current study is that a large variation of adiponectin concentrations in our DN patients. One Taiwanese study found that the +45G allele was associated with a reduced risk of obesity as it led to increased mRNA expressions in omental adipose tissue [29]. In our study, we also found that patients carrying +45 GG genotype had higher circulating adiponectin levels than GT+TT carriers, although the difference did not reach statistical

significance. However, one European study showed that +45G allele carriers had a greater risk of renal event resulting from high adiponectin concentrations in this group [26]. The *ADIPOQ* genetic mechanisms in the mRNA expression and adiponectin production remain largely unknown in patients with kidney disease.

The strength of the present study was its prospective design. The study is limited in that it had a relatively small sample size when further stratified by gender. However, the results found in males did reach 80% of power and were reproducible in the case-control analysis. Another limitation is that the genetic association found in this study may not be generalized to other ethnic groups. Larger ethnically matched studies might help clarify whether this genetic association can be found in other Asian and Chinese populations.

In conclusion, this study provides evidence that the rs2241766 (+45T>G), rs1063537, rs2241767 and rs2082940 polymorphisms in the *ADIPOQ* are associated with the progression of DN in Taiwanese male patients with T2D. Whether gender plays some roles in the association between *ADIPOQ* genetic variations and the development of DN warrants further investigations in different ethnic populations.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Table 1 Baseline clinical characteristics of 566 patients with type 2 diabetes stratified by nephropathy progression and gender

	Nephropathy			Non-Nephropathy			P-values		
	All	Male	Female	All	Male	Female	All	Male	Female
Number	144	70	74	422	193	229			
Age (y)	55.5 ± 9.0	54.7 ± 8.9	56.3 ± 9.1	55.1 ± 8.2	53.4 ± 8.4	56.6 ± 7.7	0.621	0.250	0.774
Diabetes duration (y)	5.5 ± 5.6	5.1 ± 5.3	5.9 ± 5.9	4.3 ± 5.5	3.9 ± 4.1	4.6 ± 6.3	0.021	0.084	0.114
Education≤6y (%)	95 (66.0)	39 (55.7)	56 (75.7)	213 (50.5)	60 (31.1)	153 (66.8)	0.001	<0.001	0.152
Current smoker (%)	28 (19.4)	26 (37.1)	2 (2.7)	67 (15.9)	58 (30.1)	9 (3.9)	0.323	0.276	0.624
ACEI or ARB use (%)	102 (70.8)	49 (70.0)	53 (71.6)	264 (62.6)	118 (61.1)	146 (63.8)	0.073	0.187	0.215
Body mass index (kg/m^2)	26.1 ± 4.0	26.0 ± 3.1	26.2 ± 4.8	26.0 ± 3.7	26.1 ± 3.5	26.0 ± 3.9	0.949	0.920	0.990
Systolic BP (mmHg)	130.6 ± 15.6	131.4 ± 14.9	129.8 ± 16.2	127.8 ± 15.4	127.3 ± 15.7	128.3 ± 15.1	0.076	0.057	0.519
Diastolic BP (mmHg)	81.3 ± 9.7	82.3 ± 9.6	80.3 ± 9.8	80.0 ± 10.0	80.8 ± 9.7	79.4 ± 10.1	0.173	0.240	0.480
HbA1C (%)	8.5 ± 1.8	8.5 ± 1.7	8.5 ± 1.8	8.0 ± 1.7	8.0 ± 1.8	8.0 ± 1.7	0.005	0.042	0.049
Triglyceride (mmol/L)	1.9 ± 1.4	2.1 ± 1.7	1.6 ± 0.9	1.7 ± 1.2	1.7 ± 1.3	1.7 ± 1.1	0.156	0.021	0.639
Cholesterol (mmol/L)	5.0 ± 1.0	5.1 ± 1.0	5.0 ± 0.9	4.9 ± 1.0	4.9 ± 1.1	5.0 ± 1.0	0.561	0.162	0.586
LDL cholesterol (mmol/L)	3.3 ± 0.8	3.3 ± 0.9	3.2 ± 0.8	3.2 ± 0.9	3.2 ± 0.9	3.2 ± 0.9	0.843	0.698	0.912
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	1.1 ± 0.2	1.4 ± 0.4	0.799	0.743	0.726
Creatinine (μmol/L)	67.8 ± 23.6	81.2 ± 24.9	55.3 ± 13.2	67.0 ± 18.0	76.0 ± 15.7	59.6 ± 16.4	0.806	0.232	0.024
ACR (mg/mmol)	1.2 ± 0.8	1.2 ± 0.9	1.1 ± 0.8	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.6	<0.001	<0.001	<0.001
Adiponectin (ng/ml)[1]	7.5 ± 7.0	6.3 ± 6.4	8.7 ± 7.3	6.7 ± 5.6	5.0 ± 3.6	8.1 ± 6.6	0.318	0.207	0.662

Data are presented as mean±SD or n (%). Abbreviation: ACEI: angiotensin-converting-enzyme inhibitor; ARB: angiotensin receptor blockers; ACR: urinary albumin-to-creatinine ratio.

1. Plasma adiponectin concentrations were measured cross-sectionally in the year 2008 of this cohort.

Table 2 Genotype and allele distribution of 18 *ADIPOQ* polymorphisms in type 2 diabetic patients with and without progression to nephropathy

SNPs	Location	M/m	Nephropathy (n=144)		Non-Nephropathy (n=422)				HWE
			Genotype (MM/Mm/mm)	Allele (M/m)	Genotype (MM/Mm/mm)	Allele (M/m)	P[1]	P[2]	P[3]
rs4632532 (-19166 T>C)	promoter	T/C	47/70/27 (32.6/48.6/18.8)	164/124 (56.9/43.1)	123/223/76 (29.1/52.8/18.0)	469/375 (55.6/44.4)	0.657	0.685	0.150
rs16861194 (-11426 A>G)	promoter	A/G	106/32/6 (73.6/22.2/4.2)	244/44 (84.7/15.3)	299/114/9 (70.9/27.0/2.1)	712/132 (84.4/15.6)	0.253	0.884	0.626
rs17300539 (-11391G>A)	promoter	G/A	144/0/0 (100.0/0.0/0.0)	288/0 (100.0/0.0)	422/0/0 (100.0/0.0/0.0)	844/0 (100.0/0.0)	-	-	-
rs266729 (-11377 C>G)	promoter	C/G	98/44/2 (68.1/30.6/1.4)	240/48 (83.3/16.7)	283/133/6 (67.1/31.5/1.4)	699/145 (82.8/17.2)	0.974	0.841	0.027
rs182052 (-10066 G>A)	intron 1	G/A	50/71/23 (34.7/49.3/16.0)	171/117 (59.4/40.6)	136/220/66 (32.2/52.1/15.6)	492/352 (58.3/41.7)	0.828	0.748	0.138
rs16861205 (-9215 G>A)	intron 1	G/A	107/33/4 (74.3/22.9/2.8)	247/41 (85.8/14.2)	298/113/11 (70.6/26.8/2.6)	709/135 (84.0/16.0)	0.659	0.477	0.941
rs822394 (-4120 A>C)	intron 1	C/A	106/36/2 (73.6/25.0/1.4)	248 /40 (86.1/13.9)	309/111/2 (73.2/26.3/0.5)	729/115 (86.4/13.6)	0.444	0.911	0.016
rs822396 (-3971 G>A)	intron 1	A/G	107/29/2 (77.5/21.0/1.4)	243/33 (88.0/12.0)	311/102/2 (74.9/24.6/0.5)	724/106 (87.2/12.8)	0.287	0.724	0.036
rs12495941(-2668 G>T)	intron 1	G/T	45/78/21 (31.2/54.2/14.6)	168/120 (58.3/41.7)	153/204/65 (36.3/48.3/15.4)	510/334 (60.4/39.6)	0.461	0.531	0.825
rs7627128 (-2049 C>A)	intron 1	C/A	90/46/8 (62.5/31.9/5.6)	226/62 (78.5/21.5)	253/154/15 (60.0/36.5/3.6)	660/184 (78.2/21.8)	0.406	0.923	0.149
rs2241766 (+45 T>G)	exon 2	T/G	77/57/10 (53.5/39.6/6.9)	211/77 (73.3/26.7)	206/186/30 (48.8/44.1/7.1)	598/246 (70.9/29.1)	0.614	0.434	0.168
rs1501299 (+276 G>T)	intron 2	G/T	78/57/9 (54.2/39.6/6.2)	213/75 (74.0/26.0)	221/179/22 (52.4/42.4/5.2)	621/223 (73.6/26.4)	0.785	0.899	0.062
rs2241767 (+349 A>G)	intron 2	A/G	78/57/9 (54.2/39.6/6.2)	213/75 (74.0/26.0)	212/180/30 (50.2/42.7/7.1)	604/240 (71.6/28.4)	0.712	0.434	0.324
rs3821799 (+639 T>C)	intron 2	T/C	45/72/27 (31.2/50.0/18.8)	162/126 (56.2/43.8)	139/221/62 (32.9/52.4/14.7)	499/345 (59.1/40.9)	0.513	0.393	0.086
rs3774261 (+712 A>G)	intron 2	A/G	41/72/31 (28.5/50.0/21.5)	154/134 (53.5/46.5)	121/228/73 (28.7/54.0/17.3)	470/374 (55.7/44.3)	0.503	0.514	0.052
rs6773957 (+2858 A>G)	3' UTR	A/G	40/73/31 (27.8/50.7/21.5)	153/135 (53.1/46.9)	123/224/75 (29.1/53.1/17.8)	470/374 (55.7/44.3)	0.608	0.450	0.121
rs1063537 (+3228 C>T)	3' UTR	C/T	78/57/9 (54.2/39.6/6.2)	213/75 (74.0/26.0)	212/180/30 (50.2/42.7/7.1)	604/240 (71.6/28.4)	0.712	0.434	0.324
rs2082940 (+3317 T>C)	3' UTR	C/T	76/59/9 (52.8/41.0/6.2)	211/77 (73.3/26.7)	212/181/29 (50.2/42.9/6.9)	605/239 (71.7/28.3)	0.864	0.605	0.246

Data are presented as n (%). Abbreviation: SNP: single nucleotide polymorphism; M/m: major allele/minor allele; 3'UTR: 3'untranslated region; HWE: Hardy-Weinberg equilibrium.

1. Genotype p-value and 2. Allelic p-value were examined by chi-squared tests. 3. Chi-squared test was used to test HWE for genotype frequencies in the non-nephropathy group.

Table 3 Gender stratified analysis of genotype and allele distribution of *ADIPOQ* polymorphisms in type 2 diabetic patients with and without progression to nephropathy

Gender	SNPs	Genotype	Nephropathy	Non-Nephropathy	P-value	HR (95% CI)	P-value
Male	rs2241766	GG	3 (4.3)	18 (9.3)	0.133	1.00	
		GT	30 (42.9)	97 (50.3)		1.64 (0.50-5.38)	0.413
		TT	37 (52.9)	78 (40.4)		2.47 (0.76-8.00)	0.132
		T allele	104 (74.3)	253 (65.5)	0.058	1.45 (0.99-2.12)	0.053
	rs1063537	TT	3 (4.3)	19 (9.8)	0.099	1.00	
		CT	29 (41.4)	95 (49.2)		1.72 (0.52-5.65)	0.371
		CC	38 (54.3)	79 (40.9)		2.65 (0.82-8.57)	0.105
		C allele	105 (75.0)	253 (65.5)	0.040	1.50 (1.03-2.21)	0.037
Female	rs2241766	GG	7 (9.5)	12 (5.2)	0.446	1.00	
		GT	27 (36.5)	89 (38.9)		0.63 (0.27-1.45)	0.275
		TT	40 (54.1)	128 (55.9)		0.66 (0.30-1.47)	0.306
		T allele	107 (72.3)	345 (75.3)	0.462	0.90 (0.63-1.29)	0.575
	rs1063537	TT	6 (8.1)	11 (4.8)	0.515	1.00	
		CT	28 (37.8)	85 (37.1)		0.70 (0.29-1.69)	0.429
		CC	40 (54.1)	133 (58.1)		0.66 (0.28-1.55)	0.336
		C allele	108 (73.0)	351 (76.6)	0.366	0.87 (0.60-1.25)	0.439

Data are presented as n (%) and HR (95% CI). Abbreviation: SNP: single nucleotide polymorphism.

Table 4 Gender stratified analysis of association between *ADIPOQ* polymorphisms and the progression of diabetic nephropathy under different genetic models

Gender	SNPs	Genetic models	Crude model		Adjusted model [1]	
			HR (95% CI)	P-value	HR (95% CI)	P-value
Male	rs2241766	Dominant (GT+TT vs. GG)	2.01 (0.63-6.41)	0.235	1.74 (0.55-5.57)	0.348
		Recessive (TT vs. GT+GG)	1.59 (1.00-2.55)	0.053	1.81 (1.10-2.96)	0.019
		Additive (GG/GT/TT)	1.53 (1.02-2.29)	0.038	1.66 (1.08-2.56)	0.021
		Allele (T vs. G)	1.45 (0.99-2.12)	0.053	1.50 (1.02-2.21)	0.041
	rs1063537	Dominant (CT+CC vs. TT)	2.15 (0.68-6.82)	0.196	1.90 (0.59-6.05)	0.280
		Recessive (CC vs. CT+TT)	1.64 (1.03-2.63)	0.039	1.89 (1.15-3.11)	0.013
		Additive (TT/CT/CC)	1.57 (1.05-2.35)	0.027	1.73 (1.12-2.66)	0.013
		Allele (C vs. T)	1.50 (1.03-2.21)	0.037	1.56 (1.06-2.31)	0.026
Female	rs2241766	Dominant (GT+TT vs. GG)	0.65 (0.30-1.41)	0.271	0.84 (0.36-1.98)	0.689
		Recessive (TT vs. GT+GG)	0.97 (0.61-1.52)	0.878	1.03 (0.65-1.66)	0.890
		Additive (GG/GT/TT)	0.90 (0.63-1.30)	0.573	0.99 (0.68-1.44)	0.957
		Allele (T vs. G)	0.90 (0.63-1.29)	0.575	0.99 (0.68-1.44)	0.958
	rs1063537	Dominant (CT+CC vs. TT)	0.67 (0.29-1.55)	0.354	0.91 (0.36-2.30)	0.842
		Recessive (CC vs. CT+TT)	0.89 (0.56-1.40)	0.606	0.95 (0.59-1.52)	0.822
		Additive (TT/CT/CC)	0.86 (0.60-1.25)	0.435	0.95 (0.65-1.39)	0.793
		Allele (C vs. T)	0.87 (0.60-1.25)	0.439	0.95 (0.65-1.38)	0.796

Data are presented as HR (95% CI). Additive genetic effects were modeled by defining continuous variable with levels 1, 2, and 3 corresponding to genotypes (i.e. rs2241766 was coded as 1 for GG, 2 for GT, and 3 for TT; rs1063537 was coded as 1 for TT, 2 for CT, and 3 for CC). Dominant model was coded as 1 for any genotype that contains disease allele and 0 otherwise. Recessive model was coded as 1 for the homozygous disease genotype and 0 otherwise.

1. Adjusted model: diabetes duration, education (≤6, >6 years), blood pressure (≥140/90, <140/90mmHg), HbA1C, triglyceride, and use of ACEI or ARB (yes, no) at baseline. Female patients were additionally adjusted for baseline menopause status (pre-, post-menopause).

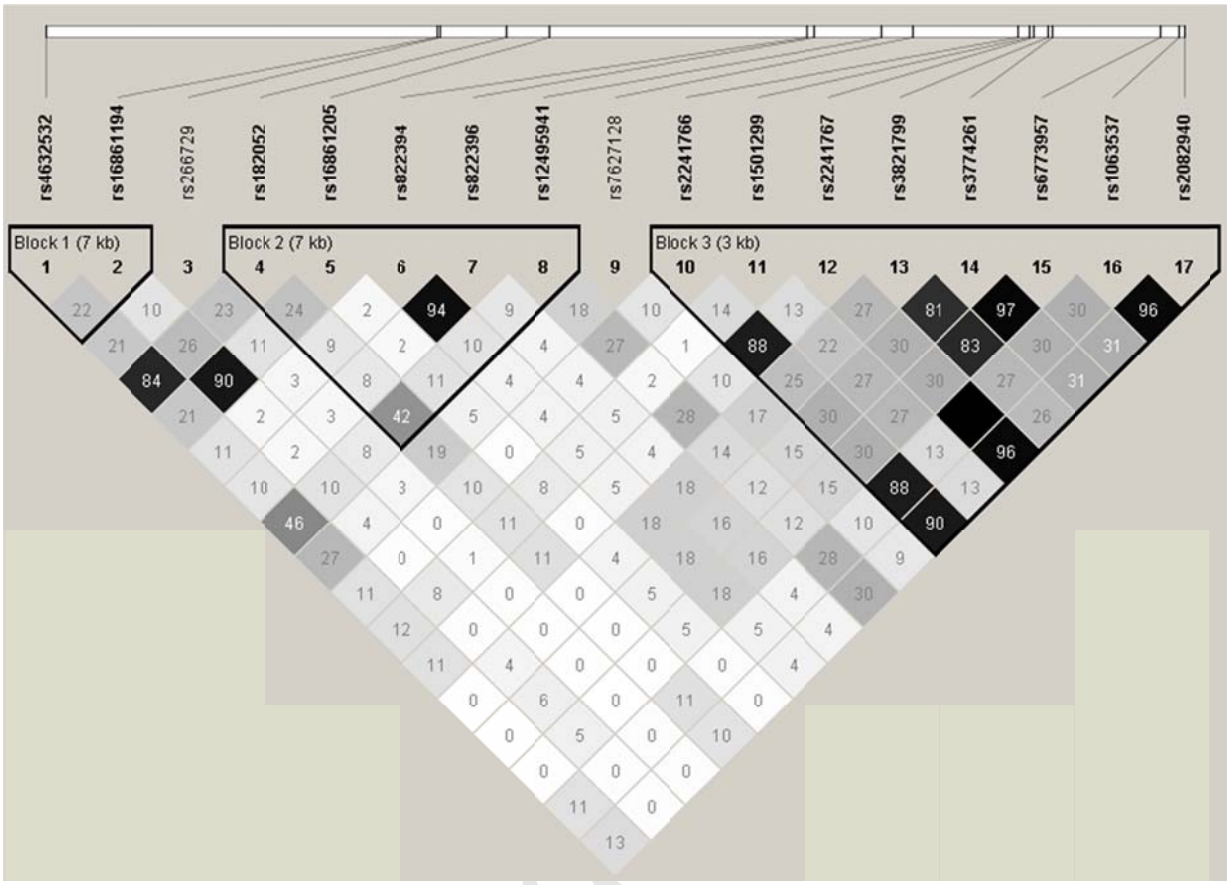

Figure 1 Position and linkage disequilibrium (LD) plot generated by the 17 *ADIPOQ* polymorphisms genotyped, with pairwise r^2 values and color scheme. Pairwise $r^2 \geq 0.8$ are shown by black color, which determined polymorphism pairs in strong LD. A haplotype block represents a region with a paucity of haplotype diversity (2-4 per block), separated by recombination hotspots.

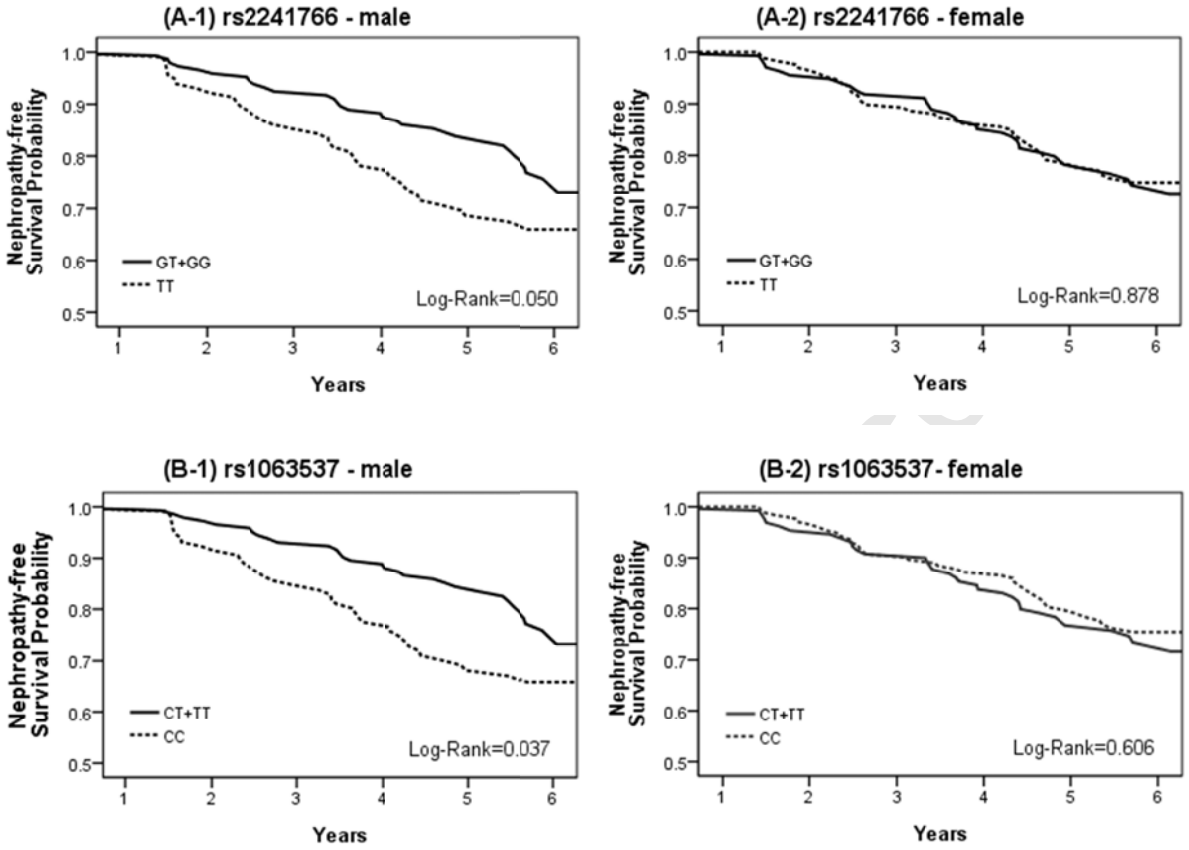

Figure 2 Estimates of diabetic nephropathy event-free survival by the Kaplan-Meier method. Survival probabilities were estimated under recessive model of *ADIPOQ* polymorphisms (A) rs2241766 and (B) rs1063537, stratified by gender.