Common variants in CNDP1 and CNDP2, and risk of nephropathy in type 2 diabetes.

Ahluwalia, Tarun; Lindholm, Eero; Groop, Leif

Published in:
Diabetologia

DOI:
10.1007/s00125-011-2178-5

2011

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Common variants in the *CNDP1* and *CNDP2* genes and risk of nephropathy in type 2 diabetes

T. S. Ahluwalia¹, E. Lindholm¹, L. C. Groop¹

¹Department of Clinical Sciences-Diabetes & Endocrinology and Lund University Diabetes Centre, Lund University, University Hospital Skane, Malmö, Sweden.

Corresponding author:
Taranveer Singh Ahluwalia, PhD
Department of Clinical Sciences- Diabetes and Endocrinology,
Lund University Diabetes Centre,
Entrance 72, Building 91, Plan 12,
Clinical Research Centre (CRC),
University Hospital Skane (UMAS),
20502, Malmo, Sweden.
Email: Tarun.Ahluwalia@med.lu.se
Phone: +46-40-391211
Fax: +46-40-391222
**Aims/Hypothesis:** Several genome-wide linkage studies have shown association of diabetic nephropathy (DN) with a locus on chromosome 18q harboring two carnosinase genes, CDNP1 and CDNP2. Carnosinase degrades carnosine (β-alanyl-L-histidine) which has been ascribed renal protective effects as a scavenger of reactive oxygen species. We aimed to investigate the putative association of genetic variants in the CNDP1 and CNDP2 genes with DN (defined either as micro or macroalbuminuria) and glomerular filtration rate (eGFR) in type 2 diabetic patients from Sweden.

**Methods:** We genotyped nine single nucleotide polymorphisms (SNPs) and one trinucleotide repeat polymorphism D18S880 (5-7 Leucine repeats) in the CNDP1 and CNDP2 genes in a case control set-up including 4,888 unrelated type 2 diabetic individuals (with and without nephropathy) from Sweden (Scania Diabetes Registry).

**Results:** Two SNPs rs2346061 in CNDP1 and rs7577 in CNDP2 were associated with increased risk of DN (rs2346061, p: 5.07 x 10^{-4}; rs7577, p: 0.021) and the latter with eGFR (β: -0.037, p: 0.014), particularly in women. A haplotype including these SNPs (C-C-G) was associated with a 3-fold increased risk of DN (OR: 2.98, 95%CI: 2.43-3.67, p<0.0001).

**Conclusions/Interpretation:** In conclusion, these data suggest that common variants in the CNDP1 and CNDP2 genes play a role in susceptibility to kidney disease in type 2 diabetes.

**Keywords:** Carnosinase, CNDP, diabetic nephropathy, type 2 diabetic nephropathy, renal function, glomerular filtration rate, genetic polymorphisms, CNDP1, CNDP2, Carnosine, haplotype.

**Abbreviations:** CNDP: Carnosine dipeptidase, DN: diabetic nephropathy, ESRD: end stage renal disease, eGFR: estimated glomerular filtration rate, SDR: Scania diabetes registry, SNP: single nucleotide polymorphism, ACR: Albumin creatinine ratio, AGEs: advanced glycation end products, UACR: urinary albumin creatinine ratio, LD: Linkage disequilibria. OR: odds ratio, CI: confidence interval
Introduction

Diabetic nephropathy (DN) is one of the most severe complications of type 1 and type 2 diabetes mellitus and the leading cause of end-stage renal disease (ESRD), and renal replacement therapy [1, 2]. DN seems to result from a complex interaction between genetic susceptibility and the diabetic environment characterized by poor glycemic control and hypertension [3-6]. About 35% of type 2 diabetes patients develop DN [6]. Familial clustering has been observed in DN, supporting the presence of a genetic component [4, 7]. Increased urinary albumin excretion is a hallmark of DN and the heritability for albuminuria has been estimated to be about 0.4 [7, 8]. Recent genome wide linkage scans in different ethnic groups have shown association of DN to a locus on chromosome 18q [9-11] which also harbours two carnosine dipeptidase genes, CDNP1 and CDNP2.

The CDNP1 and CNDP2 genes lie adjacent on chromosome 18q; CDNP1 encodes a dipeptidase that hydrolyzes the substrate L-carnosine (β-alanyl-L-histidine) specifically, while CNDP2 encodes a nonspecific dipeptidase [12]. Carnosine has been ascribed a protective role in DN by serving as a scavenger of oxygen radicals which can inhibit formation of advanced glycation end products (AGEs) [13, 14]. Jansen et al. also provided the first evidence for an association between a tri-nucleotide repeat variant (D18S880: 5-7 leucine repeats) in the signal peptide of exon 2 of the CDNP1 gene and DN in type 2 diabetes patients of European and Arab ancestry [15]. This finding was subsequently replicated in type 2 diabetes patients with DN-ESRD of European origin in USA [16]. In a follow-up study in African Americans they identified 2 SNPs in the CDNP1 and CNDP2 genes being associated with type 2 diabetes and ESRD [17]. However, several studies have not been able to replicate these findings [18, 19], nor has any association been shown between the CNDP locus and DN in type 1 diabetes [20-22]. The 5L-5L genotype of the D18S880 marker has been reported to be associated with low serum carnosinase (CNDP) concentrations in diabetics [15] making the CNDP genes interesting candidates for studying DN susceptibility. Also, in support of a role for CDNP1 and CNDP2 in DN there is differential expression of these genes in kidneys of diabetic animals [23].
Against this background of discrepant results this study was designed to explore whether there is an association between SNPs (including the repeat variant) in the \textit{CNDP} locus and DN in a large well characterized population of patients with type 2 diabetes from Southern Sweden (Scania Diabetes Registry, SDR).

\section*{Methods}

\textbf{Study Population: The Scania Diabetes Register (SDR)}

All patients were from the Scania Diabetes Registry (SDR) in Southern Sweden (Table 1). At the time of investigation, the registry included 1264 type 1 diabetes and 5123 type 2 diabetes patients with mean disease duration of 14 years (Table 1). The registry contains information on age at onset of diabetes, mode of treatment, time for starting insulin therapy as well as follow-up data on change in BMI, HbA1c, lipids, blood pressure and development of diabetic complications.

Inclusion criteria in the present study: Scandinavian origin, age at onset of diabetes \textgreater 35 years, diabetes duration of \textgreater= 10 years, C-peptide \textgreater 0.3 nmol/l, and GAD antibody negativity. Diabetes diagnosis was based upon WHO criteria with a fasting plasma glucose (FPG) \textgreater 7.0 mmol/l. In total 4,888 type 2 diabetes patients were included in the study. Diabetic patients with other kidney disease (n=35) were excluded.

Albumin excretion rate (AER) was measured from timed overnight urine collections or as albumin/creatinine ratio (ACR) in morning spot urine on at least two out of three occasions with 6 month intervals during follow up; albumin concentration in urine was determined by immunonephelometry (Beckman Instruments, CA, USA) until 1998 and thereafter by an immunoturbimetric method (Beckman Coulter, Beckman Instruments, CA, USA).

DN was subdivided into incipient (microalbuminuria) and manifest (macroalbuminuria) DN. Microalbuminuria was defined as a) AER of 20–200 $\mu$g/min in at least two timed overnight urine samples or, b) an albumine:creatinine ratio of 2.0–25 g/mol in males and 3.5–35 g/mol in females. Values above the upper limit of the definition for microalbuminuria were indicative of macroalbuminuria. Based upon this definition 880 type 2 diabetes patients were considered to have DN in the SDR (Table 1).
Renal function was estimated from serum creatinine concentrations and expressed as estimated glomerular filtration rate (eGFR). The formula for GFR (Cockroft and Gault) was (mL/min per 1.73 m2) = [(140-age in years) x weight in kg x 1.73]/(plasma creatinine in µmol/L x F x BSA), where F= 0.8 if male and F= 0.85 if female [24].

All patients gave their written informed consent and the local ethics committees approved the study.

Selection of SNPs and haplotyping

Nine SNPs and one microsatellite marker D18S880, in the CNDP1 and CNDP2 genes (chromosome 18 position: 70321335 to 70395500 bps) with a minor allele frequency (MAF) > 0.05 in Europeans were selected from the dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and HapMap (http://www.hapmap.org/) data bases. These SNPs capture 50% of variants (36 neighbouring SNPs) with a MAF > 5% at r² ≥ 0.8 from the CNDP1 gene region (Supplementary table 3; Hapmap 1000 genomes version) [25].

Genotyping

Nine SNPs (7 in CDNP1 and 2 in CNDP2) were genotyped, in 4888 patients from the SDR by an allelic discrimination method on the ABI 7900 platform (TaqMan assay, Applied Biosystems, Foster City, CA); We obtained an average genotyping success rate of 98% and a 99.9% concordance rate, based on 780 duplicate comparisons using Taq Man assays.

Genotyping for the D18S880 microsatellite marker was performed by sequence analysis using an ABI 3130x1 capillary sequencer (Applied Biosystems, Darmstadt, Germany) in all the study subjects. The primers used were, AGGCAGCTGTGTGAGGTAAC (forward) and GGGTGAGGAGAACATGCC (reverse) where the forward primer was FAM-labeled at the 5’ end (Eurofins MWG Operon, Germany) and a PCR product length of 167bp confirmed the presence of 5 CTG repeat units (five leucine codons). Random samples were sequenced later to confirm a genotyping concordance rate of 99.9%.
Statistical analyses

Data are presented as means ± SD. Non-normally distributed variables (albumin-creatinine ratio (ACR), and eGFR) were logarithmically (natural) transformed for analyses. Variables showing skewed distribution were compared using Mann–Whitney U-test. The risk of developing DN expressed as odds ratio (OR) and 95% confidence intervals (CI) were calculated by logistic regression analyses adjusted for age and sex. Genotype-phenotype correlations were assessed using linear regression analyses adjusted for age, sex, diabetes duration, HbA1c, smoking status, systolic blood pressure and BMI (where appropriate). ANOVA with the Bonferroni test as post hoc test were used to evaluate differences between means. Deviations from Hardy-Weinberg equilibrium were evaluated with a Pearson’s \( \chi^2 \) goodness-of-fit test. Correction for multiple testing was performed using the QVALUE software package (http://faculty.washington.edu/~jstorey/qvalue/). Haplotypes were reconstructed using the PHASE (2.1v; http://stephenslab.uchicago.edu/software.html) program [26]. All statistical genetic analyses were performed using an additive model (dominant and recessive models were also used) with the Statistical Package for the Social Sciences v.17.0 (SPSS, Chicago, IL), and PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) [27].

Power analysis

The study could detect a genotype RR of 1.15 with a power of at least 70% at \( P < 0.05 \). To calculate power for SNPs in our study, we used the PS program of Dupont and Plummer (available at http://www.mc.vanderbilt.edu/prevmed/ps/index.htm) [28].

Results

Characteristics of patients

Table 1 compares the clinical characteristics between the 4008 type 2 diabetes patients with normoalbuminuria and the 880 type 2 diabetes patients with DN. While there was no difference in age, the DN patients had longer duration of diabetes than normoalbuminuric type 2 diabetes
patients. The DN patients had lower eGFR, HDL, and cholesterol concentrations but, higher blood pressure, ACR, HbA1c and triglyceride concentrations than normoalbuminuric type 2 diabetes patients. Smoking (51 vs. 36%; p<0.01) and retinopathy (51 vs. 32%; p<0.01) were more common in DN than normoalbuminuric patients.

**Association between CNDP SNPs and risk of diabetic nephropathy and eGFR**

Of the genotyped SNPs and a microsatellite marker from the *CDNP genes*, rs4891558 (*CNDP2*) was monomorphic (minor allele frequency < 0.05) in this cohort and all the SNPs except one (rs12604675 from *CNDP1*) fulfilled Hardy-Weinberg equilibrium (P >0.10). The rs2346061 SNP in the *CNDP1* gene showed a significant association with DN in additive (OR: 1.25, 95%CI: 1.1-1.4, p= 5.07 x 10^{-4}; Table 2 and Figure 2) and alternate models (Table 2) but not with eGFR (Table 3).

The rs7577 SNP in the *CNDP2* gene was nominally associated with DN in additive (OR: 1.15, 95%CI: 1.02-1.3, p=0.021) and recessive models (p=0.016) (Table 2 and Figure 2). The same SNP was also associated with eGFR in all type 2 diabetes subjects with lower eGFR in CC and TC than TT genotype (rs7577) carriers (p=0.014; Table 3) particularly in women. The difference in eGFR between CC and TT/TC genotype carriers (log eGFR_{women}: CC: 4.52 ± 0.35, TC: 4.58 ± 0.34, TT: 4.59 ± 0.34, β=0.036, p=0.031; Figure 3) was associated with a higher urinary albumin creatinine ratio (UACR) (log UACR_{women}: CC: 2.01 ± 0.55, TC: 1.72 ± 0.54, TT: 1.23 ± 0.63, β=0.11, p=0.0001; Figure 4).

Table 4 shows genotype frequencies for the D18S880 microsatellite marker. As previously reported [17] we identified the 5L, 6L, or 7L leucine repeats, whereas the 4L and 8L repeats were very rare (<0.005%) and not considered for analysis. There were no significant differences in D18S880 genotype frequencies (P=0.17; df=2) between DN and type 2 diabetes patients and this marker was not associated with DN in the SDR cohort (Supplementary table 2).

The other genotyped SNPs (rs7244370, rs7239132, rs12964454, rs12456388, and rs9953129) did not show any significant association with risk of DN or eGFR (Supplementary Table 1 and Supplementary Table 2).
rs7239132 SNP of the CNDP1 gene was associated with HbA1c levels. The AA genotype carriers had significantly lower HbA1c levels as compared to CA/CC carriers (Table 3).

**Haplotype Analysis**

The three SNPs having LD values as, D’=0.98 (r²= 0.75) between rs7577 and rs7244370, D’=0.84 (r²= 0.24) between rs2346061 and rs7244370, and D’= 0.43 (r²=0.14) between rs7577 and rs2346061, were used for haplotype reconstruction to study the risk associated with different allelic combinations of the common variants. Their frequencies and risk associated with DN have been presented in Table 5.

Haplotype C-C-G (including alleles from rs7577, rs2346061 and rs7244370) was associated with a 3-fold increased risk of DN (Table 5) as well as reduced eGFR (β=-0.039; p_adjusted=0.011).

**Discussion**

The key finding of the present study was that the SNP in the 3’UTR of the CNDP2 gene (rs7577) was associated with increased risk of DN as shown by increased ACR and decreased eGFR, particularly in women. Another SNP in the CNDP1 gene (rs2346061) promoter was associated with DN. However, it did not influence eGFR. A haplotype consisting of these alleles was associated with an increased risk of DN and reduced eGFR.

The SNPs rs2346061 and rs7577 are located in the regulatory region of CNDP1 and CNDP2 and could thereby modulate carnosinase activity in the same way as reported for another SNP in this region [15]. In previous studies of African American type 2 diabetes patients [17] as well as of European type 1 diabetes patients [22], no association was seen between this SNP and DN. It was recently claimed that the association between DN and CNDP1 [29], was sex specific and restricted to females. In support of this finding, women with the CC genotype (rs7577) had reduced eGFR as compared to women with the TC/TT genotypes. This finding was further supported by 1.5 times higher albumin-creatinine ratios in women with the CC than in women with TC/TT genotypes. It may not be surprising to find gender specific differences for associations with the CNDP genes as women have been reported to have lower carnosine levels...
in muscle than men due higher serum carnosinase levels [30]. Also, in female mice carnosine levels increased > 250% in muscle after testosterone administration [31].

The D18S880 microsatellite marker has been reported to be associated with DN in some [15, 16] but not all [17, 22] studies. Jansen et al. reported for the first time that individuals homozygous for the allele with lowest number of leucine repeats (5L) were associated with lower serum carnosinase concentrations, conferring protection from nephropathy [15]. This was further supported by another study showing higher carnosinase concentrations with increasing number of Leucine repeats in COS cells [32]. Since carnosine has been ascribed anti oxidant effects and a potential inhibitor of ACE activity and advanced glycation end products [32], the activity of the enzyme carnosinase may be important in the development of nephropathy.

Our data did not support an association between the leucine repeat and DN in this cohort of Scandinavian patients with type 2 diabetes.

The next question remains whether the promoter SNP (rs2346061) in CNDP1 really is associated with DN, since there was no effect of the variant on kidney function (eGFR). This SNP was not associated with DN (proteinuria or ESRD) in Caucasian type 1 diabetes patients (11). However, in that study (11) there was a modest association between two other SNPs in the CNDP1 promoter region (rs12954438, and rs890332) and proteinuria but not ESRD, supporting the view that promoter SNPs in CNDP1 might influence albuminuria which may not be translated into a progressive deterioration of kidney function. Notably, we excluded patients with ESRD from our study. Also, none of these SNPs are in LD ($r^2=0.03$; HapMap Version 2 release 24) with rs2346061.

Further support for the view that different SNPs in the CNDP1 gene influence albuminuria and progression to ESRD comes from a study in African American type 2 diabetes patients which did not find any association between a proxy SNP rs2346061 and ESRD [17].

However, we have no evidence that these are the causal SNPs; functional studies are needed to answer the role of potential functional effects of these SNPs. In lack of such information we also tested whether haplotypes including these two SNPs would confer a stronger effect on DN than the individual SNPs.
The haplotype C-C-G in block 2 (including alleles from rs7577, rs2346061 and rs7244370) was associated with a 3-fold increased risk of DN as well as reduced eGFR. Also another study reported stronger association between haplotypes in the CNDP2 gene region and ESRD than between individual alleles and ESRD [17].

There are pros and cons of our study. While the well characterized patient groups were large enough to have a power of 75-80% to detect an association between the two key SNPSrs7577 and rs2346061 and DN, they were still underpowered for low frequency SNPs as well as for haplotypes.

In conclusion, we provide evidence for an association between a common SNP rs2346061 in the CNDP1 gene and DN. As this SNP was not associated with kidney function it is possible that it merely increases risk for albuminuria than for progression of kidney disease as other studies also have failed to demonstrate an association of this SNP with ESRD. The SNP rs7577 in the CNDP2 gene confers increased risk of nephropathy by altering the kidney function, particularly in women. A 3-allelic haplotype in the regulatory region of the CNDP genes was associated with a 3-fold increased risk of DN and reduced eGFR suggesting that other modifying SNPs are needed to increase risk of progression towards kidney dysfunction.

**Acknowledgements:**

TA is supported by a grant from the Bo and Kerstin Hjelt Diabetes Foundation ([http://www.hjeltfoundations.org/](http://www.hjeltfoundations.org/)). The study was further supported by grants from the Swedish Research Council, including a Linné grant (2008-6589) and a Strategic Research Grant (Exodiab; 2009-1039), the Heart-Lung Foundation, the Swedish Diabetes Research Foundation, the Knut and Alice Wallenberg Foundation, the Novo Nordisk Foundation, as well as European Community 7th Framework Programme grants (ENGAGE; Health-2007-201413 and an Innovative Medicines Initiative grant (SUMMIT; 2009-115006)). Special thanks to Malin Svensson for her help in genotyping D18S880 polymorphism.

**Duality of Interest**

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


[27] Purcell S, Neale B, Todd-Brown K, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559-575


Figure 1. Linkage disequilibrium (LD) analysis of nine SNPs in CNDP2 and CNDP1, measured by D'. Dark squares signify high LD values. SNP1, rs4891558; SNP 2, rs7577; SNP 3, rs2346061; SNP 4, rs7244370; SNP 5, rs7239132; SNP 6, rs12604675; SNP 7, rs12964454; SNP 8, rs12456388; SNP 9, rs9953129.
Figure 2. Logistic regression analysis depicting the odds ratios associated with the \textit{CNDP1} and \textit{CNDP2} SNPs. SNP 2, rs7577; SNP 3, rs2346061; D18S880 polymorphism; SNP 4, rs7244370; SNP 5, rs7239132; SNP 7, rs12964454; SNP 8, rs12456388; SNP 9, rs9953129. *P<0.05, **P<0.001. Age, gender, BMI, systolic blood pressure, smoking, duration of diabetes, and HbA1c are included in this model for nephropathy. Error bars depict 95\% confidence intervals.
**Figure 3.** Genotype-based association of *CNDP2* with eGFR. Estimated GFR (ml/min/1.73m²) was log transformed and expressed as mean ± SD. P value shows the comparison among rs7577 genotype by analysis of variance with Bonferroni test as *post hoc* test. NS; non significant.

**Figure 4.** The association between rs7577 genotype and urinary albumin excretion. Urine albumin-creatinine ratio (UACR). UACR values are log transformed. *P*-value shows the comparison among rs7577 genotypes by analysis of variance with Bonferroni test as *post hoc* test. NS: not significant.
**Results**

Table 1: Clinical characteristics of DN patients (type 2 diabetes with nephropathy) and type 2 diabetes (without nephropathy) in the Scania diabetes registry (SDR).

<table>
<thead>
<tr>
<th></th>
<th>Type 2 diabetes (N=4008)</th>
<th>DN (N=880)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males (%)</strong></td>
<td>55.4</td>
<td>66.4</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>54.1 ± 14.3</td>
<td>53.7 ± 11.5</td>
</tr>
<tr>
<td><strong>Duration of T2D (years)</strong></td>
<td>13.2 ± 4.4</td>
<td>17.3 ± 6.5*</td>
</tr>
<tr>
<td><strong>BMI (Kg/m²)</strong></td>
<td>28.7 ± 5.</td>
<td>30.1 ± 5.8</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>140.9 ± 25.2</td>
<td>147.7 ± 24.3**</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>79.4 ± 13.5</td>
<td>81.2 ± 12.3</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>6.8 ± 1.8</td>
<td>7.2 ± 1.6**</td>
</tr>
<tr>
<td><strong>S. Creatinine (µmol/L)</strong></td>
<td>83.5 ± 23.0</td>
<td>92.7 ± 26.2**</td>
</tr>
<tr>
<td><strong>eGFR (ml/min/1.73m²)</strong></td>
<td>103.0 ± 34.4</td>
<td>94.2 ± 33.2**</td>
</tr>
<tr>
<td><strong>Ln ACR (mg/mmol)</strong></td>
<td>0.26 ± 1.4</td>
<td>2.06 ± 1.5***</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.2***</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>5.1 ± 1.0</td>
<td>5.0 ± 0.8*</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>2.1 ± 1.4</td>
<td>2.64 ± 1.9*</td>
</tr>
<tr>
<td><strong>Smokers (%)</strong></td>
<td>36.1</td>
<td>51.0**</td>
</tr>
<tr>
<td><strong>Retinopathy (%)</strong></td>
<td>32.1</td>
<td>51.4**</td>
</tr>
</tbody>
</table>

Values are Mean ± standard deviation; *P<0.05, **P<0.01 and ***P<0.001 vs. T2D; SBP and DBP: Systolic and diastolic blood pressure; eGFR: estimated glomerular filtration rate (baseline); Ln ACR: logarithmic albumin creatinine ratio; Type 2 diabetics (normoalbuminuria); DN: (diabetic nephropathy)
Table 2. Risk of DN predicted by various genotypic models for rs2346061 and rs7577 in the study subjects.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genotypic Association</th>
<th>Dominant</th>
<th>Additive</th>
<th>Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td>OR (95%CI)</td>
<td>P value</td>
</tr>
<tr>
<td>rs2346061</td>
<td></td>
<td>2.7E-4</td>
<td>1.62 (1.24-2.1)</td>
<td>5.07E-4</td>
</tr>
<tr>
<td>rs7577</td>
<td></td>
<td>0.43</td>
<td>1.10 (0.86-1.41)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Results are shown in all subjects: 880 DN cases, and 4,008 type 2 diabetes (normoalbuminuria) controls; P values <0.05 are shown in bold; P values for the models have been adjusted for confounding factors including age, gender, BMI, systolic blood pressure, smoking, duration of diabetes, and HbA1c.
Table 3: Effects of CNDP SNPs on kidney function (eGFR) and HbA1c in the study population.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>rs7577*</th>
<th>Additive model</th>
<th></th>
<th>rs2346061</th>
<th></th>
<th>rs7239132*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>TC</td>
<td>CC</td>
<td>β</td>
<td>SE</td>
<td>CC</td>
<td>β</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>4002</td>
<td>101.7 ±</td>
<td>99.6 ±</td>
<td>97.3 ±</td>
<td>-0.037</td>
<td>0.008</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.5</td>
<td>34.1</td>
<td>34.0</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>3756</td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
<td>6.92 ±</td>
<td>6.90 ±</td>
<td>6.77 ±</td>
<td>-0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7</td>
<td>1.7</td>
<td>1.6</td>
<td>1.6</td>
<td>1.7</td>
<td>1.6</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are Mean± SD, *P<0.05, β and SE from linear regression analysis adjusted for age, sex, BMI, duration of diabetes and denote the effect size of each effect-allele (additive model) on eGFR. eGFR: estimated glomerular filtration rate.
Table 4. The genotype frequencies for D18S880 repeat variant in Scania Diabetes Register. DN= type 2 diabetes with nephropathy (cases); type 2 diabetes without nephropathy (controls); 5L, XL: denotes 5, and X (= 6, or 7) leucine repeats. $\chi^2$ (df=2): 3.59 (p=0.17 for genotype frequencies).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5L-5L</td>
</tr>
<tr>
<td>DN (n=820)</td>
<td>0.356</td>
</tr>
<tr>
<td>Type 2 diabetes (n=4725)</td>
<td>0.387</td>
</tr>
</tbody>
</table>
Table 5: Haplotype frequencies of type 2 diabetes patients (with and without nephropathy) and risk associated with DN. The haplotype includes three SNPs (rs7577 (T>C)- rs2346061 (A>C)- rs7244370 (G>T)).

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Type 2 diabetes (normo)</th>
<th>DN</th>
<th>OR (95%CI)</th>
<th>P unadjusted</th>
<th>P adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>2n=4188</td>
<td>Frequency</td>
<td>2n=1070</td>
<td></td>
</tr>
<tr>
<td>T-A-G</td>
<td>0.46</td>
<td>1922</td>
<td>0.48</td>
<td>522</td>
<td>1.08 (0.95-1.24)</td>
</tr>
<tr>
<td>C-A-G*</td>
<td>0.20</td>
<td>825</td>
<td>0.16</td>
<td>175</td>
<td>0.78 (0.65-0.93)</td>
</tr>
<tr>
<td>C-C-G**</td>
<td>0.061</td>
<td>253</td>
<td>0.16</td>
<td>175</td>
<td>2.98 (2.43-3.67)</td>
</tr>
<tr>
<td>T-C-G**</td>
<td>0.16</td>
<td>674</td>
<td>0.093</td>
<td>100</td>
<td>0.52 (0.42-0.65)</td>
</tr>
<tr>
<td>T-A-T</td>
<td>0.065</td>
<td>270</td>
<td>0.089</td>
<td>99</td>
<td>1.45 (1.14-1.84)</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.001; N: Number of alleles. P adjusted: after multiple testing corrections