Diabetologia (2011) 54:2295–2302
DOI 10.1007/s00125-011-2178-5

ARTICLE

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

**T. S. Ahluwalia · E. Lindholm · L. C. Groop**

Received: 20 October 2010 / Accepted: 13 April 2011 / Published online: 15 May 2011


**Abstract**
*Aims/hypothesis* Several genome-wide linkage studies have shown an association between diabetic nephropathy and a locus on chromosome 18q harbouring two carnosinase genes, *CNDP1* and *CNDP2*. Carnosinase degrades carnosine (β-alanyl-L-histidine), which has been ascribed a renal protective effect as a scavenger of reactive oxygen species. We investigated the putative associations of genetic variants in *CNDP1* and *CNDP2* with diabetic nephropathy (defined either as micro- or macroalbuminuria) and estimated GFR in type 2 diabetic patients from Sweden.
*Methods* We genotyped nine single nucleotide polymorphisms (SNPs) and one trinucleotide repeat polymorphism (D18S880, five to seven leucine repeats) in *CNDP1* and *CNDP2* in a case–control set-up including 4,888 unrelated type 2 diabetic patients (with and without nephropathy) from Sweden (Scania Diabetes Registry).
*Results* Two SNPs, rs2346061 in *CNDP1* and rs7577 in *CNDP2*, were associated with an increased risk of diabetic nephropathy (rs2346061 $p=5.07\times10^{-4}$; rs7577 $p=0.021$). The latter was also associated with estimated GFR ($\beta=-0.037$, $p=0.014$), particularly in women. A haplotype including these SNPs (C-C-G) was associated with a threefold increased risk of diabetic nephropathy (OR 2.98, 95% CI 2.43–3.67, $p<0.0001$).
*Conclusions/interpretation* These data suggest that common variants in *CNDP1* and *CNDP2* play a role in susceptibility to kidney disease in patients with type 2 diabetes.

**Keywords** Carnosinase · Carnosine · CNDP · CNDP1 · CNDP2 · Diabetic nephropathy · Genetic polymorphisms · GFR · Haplotype · Type 2 diabetes

**Abbreviations**

| | |
|---|---|
| ACR | Albumin : creatinine ratio |
| CNDP | Carnosine dipeptidase |
| ESRD | End-stage renal disease |
| LD | Linkage disequilibrium |
| MAF | Minor allele frequency |
| SDR | Scania Diabetes Registry |
| SNP | Single nucleotide polymorphism |
| UACR | Urinary albumin creatinine ratio |

## Introduction

Diabetic nephropathy 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]. Diabetic nephropathy results from a complex interaction between genetic susceptibility and the diabetic environment characterised by poor glycaemic control and hypertension [3–6].

About 35% of type 2 diabetic patients develop diabetic nephropathy [6]. Familial clustering has been observed, supporting the presence of a genetic component [4, 7]. Increased urinary albumin excretion is a hallmark of diabetic nephropathy and heritability for albuminuria has

**Electronic supplementary material** The online version of this article (doi:10.1007/s00125-011-2178-5) contains supplementary material, which is available to authorised users.

T. S. Ahluwalia (✉) · E. Lindholm · L. C. Groop
Department of Clinical Sciences—Diabetes and Endocrinology, Lund University Diabetes Centre, Clinical Research Centre (CRC), University Hospital Skane (UMAS), 20502, Malmo, Sweden
e-mail: Tarun.Ahluwalia@med.lu.se

been estimated to be about 0.4 [7, 8]. Recent genome-wide linkage scans in different ethnic groups have shown an association between diabetic nephropathy and a locus on chromosome 18q [9–11], which also harbours two carnosine dipeptidase (CNDP) genes, *CNDP1* and *CNDP2*.

*CNDP1* and *CNDP2* lie adjacent to each other on chromosome 18q. The former encodes a dipeptidase that hydrolyses the substrate L-carnosine (β-alanyl-L-histidine) specifically, while *CNDP2* encodes a non-specific dipeptidase [12]. Carnosine has been ascribed a protective role in diabetic nephropathy, since it serves as a scavenger of oxygen radicals and thus can inhibit formation of AGEs [13, 14].

Janssen et al. provided the first evidence of an association between a tri-nucleotide repeat variant (D18S880: five to seven leucine repeats) in the signal peptide of exon 2 of *CNDP1* and diabetic nephropathy in type 2 diabetic patients of European and Arab ancestry [15]. This finding was subsequently replicated in type 2 diabetic patients with diabetic nephropathy and/or ESRD of European origin in the USA [16]. In a follow-up study in African-Americans the same team identified two single nucleotide polymorphism (SNPs) in *CNDP1* and *CNDP2* that were 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 diabetic nephropathy in type 1 diabetes [20–22]. The 5L-5L genotype of the D18S880 marker has been reported to be associated with low serum CNDP concentrations in diabetic patients [15], making the CNDP genes interesting candidates for diabetic nephropathy susceptibility. The possibility that *CNDP1* and *CNDP2* play a role in diabetic nephropathy is supported by a finding that these genes are differentially expressed in kidney of animal models of diabetes [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 CNDP locus and diabetic nephropathy in a large well characterised population of patients with type 2 diabetes from southern Sweden (Scania Diabetes Registry [SDR]).

## Methods

*Study population: The Scania Diabetes Registry* All patients were from the SDR in southern Sweden (Table 1). At the time of investigation, the registry included 1264 type 1 diabetes and 5123 type 2 diabetic patients with mean disease duration of 14 years (Table 1). The registry contains information on age at onset of diabetes, mode of treatment and time insulin therapy was started, as well as follow-up data on change in BMI, $HbA_{1c}$, lipids, blood pressure and development of diabetic complications.

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

Albumin excretion rate was measured from timed overnight urine collections or as albumin : creatinine ratio (ACR) in morning spot urine tests on at least two of three occasions at 6 month intervals during follow-up. Albumin

**Table 1** Clinical characteristics of type 2 diabetic patients with and without nephropathy in the SDR

| Characteristic | Diabetic nephropathy | No nephropathy |
|---|---|---|
| *n* | 880 | 4,008 |
| Men (%) | 66.4 | 55.4 |
| Age (years) | 53.7±11.5 | 54.1±14.3 |
| Duration of diabetes (years) | 17.3±6.5* | 13.2±4.4 |
| BMI (kg/m$^2$) | 30.1±5.8 | 28.7±5 |
| Systolic BP (mmHg) | 147.7±24.3** | 140.9±25.2 |
| Diastolic BP (mmHg) | 81.2±12.3 | 79.4±13.5 |
| $HbA_{1c}$ (%) | 7.2±1.6** | 6.8±1.8 |
| Serum creatinine (μmol/l) | 92.7±26.2** | 83.5±23.0 |
| eGFR (ml min$^{-1}$ 1.73 m$^{-2}$) | 94.2±33.2** | 103.0±34.4 |
| Ln ACR (mg/mmol) | 2.06±1.5*** | 0.26±1.4 |
| HDL (mmol/l) | 1.1±0.2*** | 1.2±0.3 |
| Cholesterol (mmol/l) | 5.0±0.8* | 5.1±1.0 |
| Triacylglycerol (mmol/l) | 2.64±1.9* | 2.1±1.4 |
| Smokers (%) | 51.0** | 36.1 |
| Retinopathy (%) | 51.4** | 32.1 |

Values are mean±SD, unless indicated otherwise

eGFR: estimated GFR (baseline); Ln ACR, logarithmically transformed ACR

*$p$<0.05, **$p$<0.01 and ***$p$< 0.001 vs type 2 diabetic patients without diabetic nephropathy

concentration in urine was determined by immunonephelometry (Beckman Instruments, Brea, CA, USA) until 1998 and thereafter by an immunoturbimetric method (Beckman Coulter, Beckman Instruments, Brea, CA, USA).

Diabetic nephropathy was subdivided into incipient (microalbuminuria) and manifest (macroalbuminuria) diabetic nephropathy. Microalbuminuria was defined as: (1) AER 20–200 μg/min in at least two timed overnight urine samples; or (2) ACR 2.0–25 g/mol in men and 3.5–35 g/mol in women. Values above the upper limit of the definition for microalbuminuria were indicative of macroalbuminuria. Based upon this definition, 880 type 2 diabetic patients were considered to have diabetic nephropathy in the SDR (Table 1).

Renal function was estimated from serum creatinine concentrations and expressed as estimated GFR. The formula for GFR (Cockroft and Gault) was: (ml $min^{-1}$ 1.73 $m^{-2}$)=([140−age in years]×weight in kg×1.73)/(plasma creatinine in μmol/l×$F$×BSA), where $F$ is 0.8 in men and 0.85 in women [24].

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

*Selection of SNPs and haplotyping* We selected nine SNPs and one microsatellite marker (D18S880) in *CNDP1* and *CNDP2* (chromosome 18 position: 70321335 to 70395500 bps, accessed 23 January 2010) (Fig. 1) with a minor allele frequency (MAF) >0.05 in Europeans. Selection was from the dbSNP (www.ncbi.nlm.nih.gov/snp/) and HapMap (www.hapmap.org/) databases. These SNPs capture 50% of variants (36 neighbouring SNPs) with MAF>5% at $r^2$≥0.8 from the *CNDP1* gene region (HapMap 1000 genomes version) [25] (see also electronic supplementary material [ESM] Table 1).

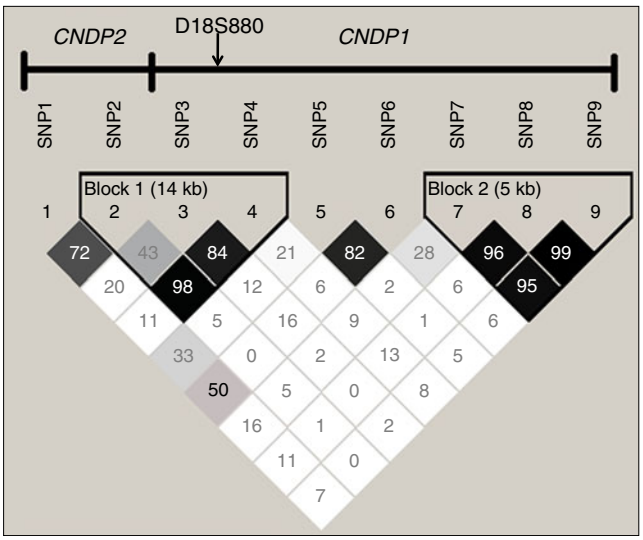


**Fig. 1** LD analysis of nine SNPs in *CNDP2* and *CNDP1*, measured by $D'$. Dark squares, high LD values. SNP1, rs4891558; SNP 2, rs7577; SNP 3, rs2346061; SNP 4, rs7244370; SNP 5, rs7239132; SNP 6, rs12604675; SNP 7, rs12964454; SNP 8, rs12456388; SNP 9, rs9953129

*Genotyping* We genotyped nine SNPs (seven in *CNDP1*, two in *CNDP2*) in 4,888 patients from the SDR using an allelic discrimination method on the ABI 7900 platform (TaqMan assay; Applied Biosystems, Foster City, CA, USA). 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 in all study participants by sequence analysis using a capillary sequencer (ABI 3130xl; Applied Biosystems, Darmstadt, Germany). The primers used were: AGG CAGCTGTGTGAGGTAAC (forward) and GGGTGAG GAGAACATGCC (reverse), where the forward primer was FAM-labelled at the 5′ end (Eurofins MWG Operon, Edersberg, Germany) and a PCR product length of 167 bp confirmed the presence of five 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 (ACR, and estimated GFR) were logarithmically (natural) transformed for analyses. Variables showing skewed distribution were compared using the Mann–Whitney $U$-test. The risk of developing diabetic nephropathy expressed as OR and 95% CI was 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, $HbA_{1c}$, smoking status, systolic blood pressure and BMI (where appropriate). ANOVA with Bonferroni's 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 QVALUE software package (http://genomics.princeton.edu/storeylab/qvalue/accessed 10 August2010). Haplotypes were reconstructed using PHASE (version 2.1; http://stephenslab.uchicago.edu/software.html, accessed 10 August 2010) [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 version 17.0 (SPSS, Chicago, IL, USA) and PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) [27].

*Power analysis* The study was able to 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

**Table 2** Risk of diabetic nephropathy predicted by various genotypic models for rs2346061 and rs7577 in the study participants

| Marker | Genotypic association | | | | | |
|---|---|---|---|---|---|---|
| | Dominant | | Additive | | Recessive | |
| | *p* value | OR (95% CI) | *p* value | OR (95% CI) | *p* value | OR (95% CI) |
| rs2346061 | $2.7\times10^{-4}$* | 1.62 (1.24–2.1) | $5.07\times10^{-4}$* | 1.25 (1.09–1.43) | 0.0038* | 0.8 (0.69–0.93) |
| rs7577 | 0.43 | 1.10 (0.86–1.41) | 0.021* | 1.15 (1.02–1.29) | 0.016* | 0.83 (0.71–0.96) |

Results are shown for all participants, i.e. 880 diabetic nephropathy cases and 4,008 normoalbuminuric type 2 diabetic controls

The *p* values for the models are adjusted for confounding factors including age, sex, BMI, systolic blood pressure, smoking, duration of diabetes and $HbA_{1c}$

*$p<0.05$

program of Dupont and Plummer (available at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize, accessed 24 January 2010) [28].

## Results

*Characteristics of patients* Table 1 compares the clinical characteristics of the 4,008 type 2 diabetic patients with normoalbuminuria with those of the 880 type 2 diabetic patients with diabetic nephropathy. While there was no difference in age, the diabetic nephropathy patients had longer duration of diabetes than normoalbuminuric counterparts. The diabetic nephropathy patients had lower estimated GFR, HDL and cholesterol concentrations, but higher blood pressure, ACR, $HbA_{1c}$ and triacylglycerol concentrations than normoalbuminuric type 2 diabetic patients. Smoking (51% vs 36%; $p<0.01$) and retinopathy (51% vs 32%; $p<0.01$) were more common in diabetic nephropathy than normoalbuminuric patients.

*Association between CNDP SNPs and risk of diabetic nephropathy and estimated GFR* Of the genotyped SNPs and microsatellite marker from the CNDP genes, only rs4891558 (*CNDP2*) was monomorphic (MAF<0.05) in this cohort; moreover, all SNPs except rs12604675 from *CNDP1*) were in Hardy–Weinberg equilibrium ($p>0.10$). The rs2346061 SNP in *CNDP1* showed a significant association with diabetic nephropathy in additive (OR 1.25, 95% CI 1.1–1.4, $p=5.07\times10^{-4}$) (Table 2 and Fig. 2) and alternate models (Table 2), but not with estimated GFR (Table 3).

The rs7577 SNP in *CNDP2* was nominally associated with diabetic nephropathy in additive (OR 1.15, 95% CI 1.02–1.3, $p=0.021$) and recessive models ($p=0.016$) (Table 2 and Fig. 2). The same SNP was also associated with estimated GFR in all type 2 diabetes participants with lower estimated GFR, and CC and TC compared with TT genotypes (rs7577) ($p=0.014$) (Table 3). This was particularly the case in women. The difference in estimated GFR 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; $\beta=0.036$, $p=0.031$) (Fig. 3) was associated with a higher urinary ACR (UACR) (log $UACR_{women}$ CC 2.01±0.55, TC 1.72±0.54, TT 1.23±0.63; $\beta=0.11$, $p=0.0001$) (Fig. 4).

Table 4 shows genotype frequencies for the D18S880 microsatellite marker. We identified the 5L, 6L or 7L leucine repeats, whereas the 4L and 8L repeats were very rare (<0.005%) and thus not considered for analysis. There were no significant differences in D18S880 genotype frequencies ($p=0.17$; $df=2$) between diabetic nephropathy and type 2 diabetic patients, and this marker was not associated with diabetic nephropathy in the SDR cohort (ESM Table 2, ESM Table 3).

The other genotyped SNPs (rs7244370, rs7239132, rs12964454, rs12456388 and rs9953129) did not show

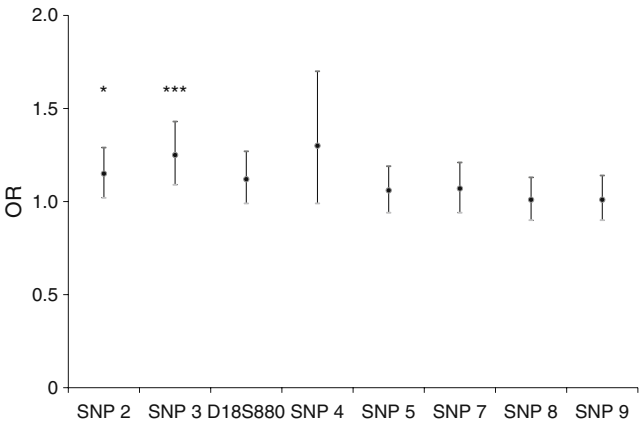


**Fig. 2** ORs (95% CIs, error bars) for diabetic nephropathy vs type 2 diabetes (normoalbuminuria) patients associated with *CNDP1* and *CNDP2* SNPs. *$p<0.05$, ***$p<0.001$ for the logistic regression analysis. Age, sex, BMI, systolic blood pressure, smoking, duration of diabetes and $HbA_{1c}$ were included in this model for nephropathy. SNP 2, rs7577; SNP 3, rs2346061; D18S880 polymorphism; SNP 4, rs7244370; SNP 5, rs7239132; SNP 7, rs12964454; SNP 8, rs12456388; SNP 9, rs9953129

**Table 3** Effects of CNDP SNPs on kidney function (estimated GFR) and $HbA_{1c}$ in the study population

| Variables per SNP | eGFR (ml $min^{-1}$ 1.73 $m^{-2}$) | $HbA_{1c}$ (%) |
|---|---|---|
| rs7577* ($n$=4002) | | |
| TT | 101.7±35.5 | – |
| TC | 99.6±34.1 | – |
| CC | 97.3±34.0 | – |
| Additive model | | |
| $\beta$ | −0.037 | – |
| SE | 0.008 | – |
| $p$ value | 0.014 | – |
| rs2346061 ($n$=4,002) | | |
| AA | 101.2±35.1 | – |
| AC | 98.7±34.3 | – |
| CC | 100.6±33.0 | – |
| Additive model | | |
| $\beta$ | −0.024 | – |
| SE | 0.009 | – |
| $p$ value | 0.09 | – |
| rs7239132* ($n$=3,756) | | |
| CC | – | 6.92±1.7 |
| CA | – | 6.90±1.7 |
| AA | – | 6.77±1.6 |
| Additive model | | |
| $\beta$ | – | −0.025 |
| SE | – | 0.008 |
| $p$ value | – | 0.024 |

Data are mean±SD

$p$ values of $p<0.05$, $\beta$ and SE from linear regression analysis were adjusted for age, sex, BMI and duration of diabetes, and denote the effect size of each effect-allele (additive model) on estimated GFR

eGFR, estimated GFR

*$p<0.05$

any significant association with risk of diabetic nephropathy or estimated GFR (ESM Table 2, ESM Table 3).

rs7239132 SNP in *CNDP1* was associated with $HbA_{1c}$ levels. The AA genotype carriers had significantly lower $HbA_{1c}$ levels than CA/CC carriers (Table 3).

*Haplotype analysis* The three SNPs with linkage disequilibrium (LD) values $D'$=0.98 ($r^2$=0.75) between rs7577 and rs7244370, $D'$=0.84 ($r^2$=0.24) between rs2346061 and rs7244370, and $D'$=0.43 ($r^2$=0.14) between rs7577and 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 diabetic nephropathy are presented in Table 5.

Haplotype C-C-G (including alleles from rs7577, rs2346061 and rs7244370) was associated with a threefold increased risk of diabetic nephropathy (Table 5) as well as reduced estimated GFR ($\beta$=−0.039; $p$=0.011, adjusted).

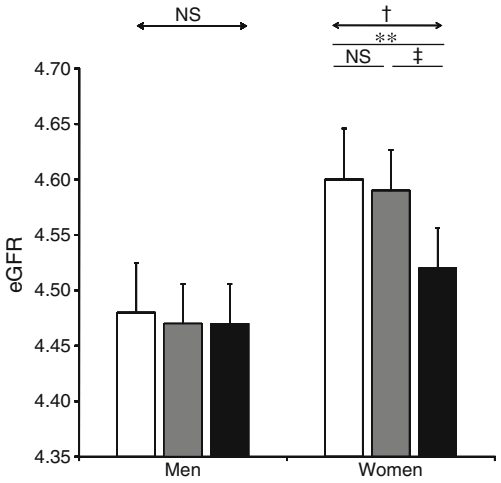


**Fig. 3** Genotype-based association between *CNDP2* and estimated GFR (eGFR). Estimated GFR (ml $min^{-1}$ 1.73 $m^{-2}$) was log-transformed and expressed as mean±SD. **$p<0.01$, $^{\dagger}p<0.031$ and $^{\ddagger}p<0.04$ for comparison in rs7577 genotype by ANOVA with Bonferroni's test as post hoc test. White bars, T/T; grey bars, T/C; black bars, C/C

## Discussion

The key finding of the present study was that the SNP in the 3′ untranslated region of *CNDP2* (rs7577) was associated with increased risk of diabetic nephropathy as shown by increased ACR and decreased estimated GFR,

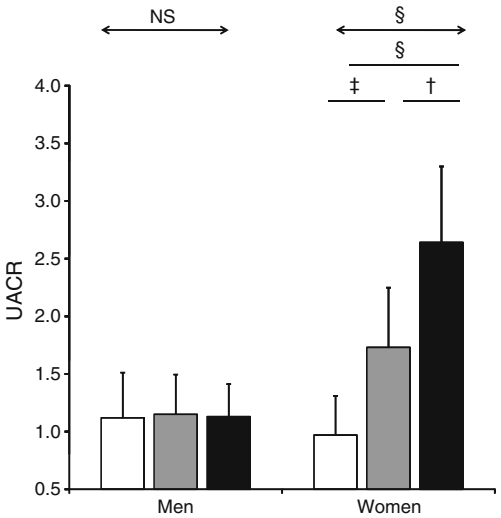


**Fig. 4** The association between rs7577 genotype and urinary albumin excretion measured as urinary ACR (UACR). Urinary ACR values were log-transformed. $^{\dagger}p$=0.04, $^{\ddagger}p$=0.004 and $^{\S}p$=0.0001 for comparison in rs7577 genotypes by ANOVA with Bonferroni's test as post hoc test. White bars, T/T; grey bars, T/C; black bars, C/C

**Table 4** The genotype frequencies for D18S880 repeat variant in SDR

| Groups | Genotype frequencies | | |
|---|---|---|---|
| | 5L-5L | 5L-XL | XL-XL |
| Diabetic nephropathy[a] | 0.356 | 0.401 | 0.243 |
| Type 2 diabetes[b] | 0.387 | 0.398 | 0.214 |

$\chi^2$ ($df$=2): 3.59 ($p$=0.17 for genotype frequencies)

5L, 5 leucine repeats; XL, 6 or 7 leucine repeats

[a] Type 2 diabetes with nephropathy, $n$=820; [b] type 2 diabetes without nephropathy (controls), $n$=4,725

particularly in women. Another SNP in the *CNDP1* (rs2346061) promoter was associated with diabetic nephropathy. However, it did not influence estimated GFR. A haplotype consisting of these alleles was associated with increased risk of diabetic nephropathy and reduced estimated GFR.

The SNPs rs2346061 and rs7577are 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 diabetic patients [17], as well as of European type 1 diabetic patients [22], no association was seen between this SNP and diabetic nephropathy. It was recently claimed that the association between diabetic nephropathy and *CNDP1*, was sex-specific [29] and restricted to women. In support of this finding, women with the CC genotype (rs7577) had reduced estimated GFR as compared with women with the TC/TT genotypes. This finding was further supported by 1.5-fold higher ACRs in women with the CC than in those with TC/TT genotypes. It is not surprising to find sex-specific differences for associations with the CNDP genes, as women have lower carnosine levels in muscle than men due to their 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 diabetic nephropathy in some [15, 16] but not all [17, 22] studies. Janssen et al. reported for the first time that in individuals homozygous for the allele with the lowest number of leucine repeats (5L), this was 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 is a potential inhibitor of ACE activity and AGEs [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 diabetic nephropathy in this cohort of Scandinavian patients with type 2 diabetes.

The next question is whether the promoter SNP (rs2346061) in *CNDP1* really is associated with diabetic nephropathy, since we observed no effect of this variant on kidney function (estimated GFR). This SNP was not associated with diabetic nephropathy (proteinuria or ESRD) in white type 1 diabetic patients [11]. However, in that study [11] there was a modest association between two other SNPs in the *CNDP1* promoter region (rs12954438, rs890332) and proteinuria but not ESRD, supporting the view that promoter SNPs in *CNDP1* might influence albuminuria but that this may not translate 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 *CNDP1* influence albuminuria and progression to ESRD comes from a study in African-American type 2 diabetic 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 define the role of

**Table 5** Haplotype frequencies in type 2 diabetic patients (with and without nephropathy) and risk associated with diabetic nephropathy

| Haplotype | No nephropathy | | Diabetic nephropathy | | Risk | | |
|---|---|---|---|---|---|---|---|
| | Frequency | 2$n$=4,188[a] | Frequency | 2n=1070[a] | OR (95% CI) | $p$ value (unadjusted) | $p$ value (adjusted)[b] |
| T-A-G | 0.46 | 1922 | 0.48 | 522 | 1.08 (0.95–1.24) | 0.21 | 0.45 |
| C-A-G | 0.20 | 825 | 0.16 | 175 | 0.78 (0.65–0.93) | 0.0065 | 0.019 |
| C-C-G | 0.061 | 253 | 0.16 | 175 | 2.98 (2.43–3.67) | <0.0001 | <0.0001 |
| T-C-G | 0.16 | 674 | 0.093 | 100 | 0.52 (0.42–0.65) | <0.0001 | <0.0001 |
| T-A-T | 0.065 | 270 | 0.089 | 99 | 1.45 (1.14–1.84) | 0.022 | 0.06 |

The haplotype includes three SNPs (rs7577 [T>C]–rs2346061 [A>C]–rs7244370 [G>T])

[a] 2$n$ = number of alleles; [b] adjusted after multiple testing corrections

potential functional effects of these SNPs. Lacking such information, we also tested whether haplotypes including these two SNPs conferred a stronger effect on diabetic nephropathy than the individual SNPs.

We found that the haplotype C-C-G in block 2 (including alleles from rs7577, rs2346061 and rs7244370) was associated with a threefold increased risk of diabetic nephropathy as well as reduced estimated GFR. Another study has also reported stronger association between haplotypes in the *CNDP2* region and ESRD than between individual alleles and ESRD [17].

Our study has some pros and cons. Thus while the well characterised patient groups were large enough to have a power of 75–80% to detect an association between the two key SNPs rs7577 and rs2346061 and diabetic nephropathy, our study was still underpowered for low frequency SNPs, as well as for haplotypes.

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

**Acknowledgements** T. S. Ahluwalia is supported by a grant from the Bo and Kerstin Hjelt Diabetes Foundation (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, and European Community 7th Framework Programme grants (ENGAGE Health-2007-201413 and an Innovative Medicines Initiative grant: SUMMIT 2009-115006)). Special thanks go to M. Svensson for her help in genotyping the D18S880 polymorphism.

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

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