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# Association of variants in the carnosine peptidase 1 gene (*CNDP1*) with diabetic nephropathy in American Indians

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## Abstract

*CNDP1* is located on 18q22.3, where linkage with diabetic nephropathy has been observed in several populations, including Pima Indians. However, evidence for association between *CNDP1* alleles and diabetic nephropathy is equivocal and population-dependent. This study investigated *CNDP1* as a candidate for diabetic kidney disease in Pima Indians. Nineteen tag single nucleotide polymorphisms spanning the *CNDP1* locus were selected using genotype data from Chinese individuals in the HapMap resource along with 2 variants previously associated with diabetic nephropathy. All variants were genotyped in 3 different samples including a diabetic end-stage renal disease (ESRD) case-control study, a family-based study of diabetic individuals who participated in the linkage study for nephropathy, and a cohort of diabetic individuals in whom longitudinal measures of glomerular filtration rates (GFR) were performed. There was no statistically significant evidence for association with diabetic ESRD. However, nominal evidence for association was found in the family study, where markers rs12957330 (Odds ratio [OR]=0.29 per copy of G allele; p=0.04) and rs17817077 (OR=0.46 per copy of G allele; p=0.05) were associated with diabetic nephropathy. In addition, markers rs12964454, rs7244647, and rs7229005 were associated with changes in GFR (−8.5 ml/min per copy of the G allele; p=0.04; 18.8 ml/min per copy of the C allele; p=0.03; and −13.4 ml/min per copy of the C allele; p=0.001, respectively). These findings provide nominal evidence supporting a role between *CNDP1* variants and diabetic kidney disease.

### Keywords

kidney disease; single nucleotide polymorphism; proteinuria



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## 1. Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in the US. In 2007, diabetes was the cause of ESRD in 54% of new patients [1], and like type 2 diabetes mellitus (T2DM), diabetic nephropathy disproportionately affects ethnic minority groups, including American Indians [2, 3]. Glycemic control, familial clustering and genetic determinants play an important role in development of diabetic kidney disease [4-7].

Carnosine dipeptidase 1 is the rate-limiting enzyme in the hydrolysis of carnosine into β-alanine and L-histidine. The gene encoding carnosine dipeptidase 1 is *CNDP1*, which is located on chromosome 18q22.3, where linkage for diabetic nephropathy has been reported [8-10]. An allelic variant ($[CTG]_5$) of a leucine repeat in exon 2 of the gene, also referred to as the "Mannheim allele" (*D18S880*), is observed at greater frequency in diabetic individuals without kidney disease in some populations [11, 12] and is also associated with lower levels of serum enzyme activity [12, 13]. However, this variant was not associated with nephropathy in African Americans with T2DM; instead, different haplotypes within the *CNDP1-CNDP2* locus were associated with the disease in this population [14, 15]. Carnosine inhibits non-enzymatic glycation and prevents both aldehyde-produced protein cross-linking and extracellular matrix accumulation due to hyperglycemia [16]. These genetic findings, along with the biological evidence suggesting that the carnosine pathway maintains kidney function in hyperglycemia, are consistent with the possibility that *CNDP1* variants modulate susceptibility to diabetic nephropathy.

With prior studies demonstrating linkage of diabetic nephropathy in Pima Indians on 18q in the region harboring *CNDP1* [10], we hypothesized that variants in the gene may affect susceptibility to nephropathy in this population. The aim of the present study, therefore, was to assess association between *CNDP1* variants and kidney disease among Pima Indians with T2DM.

## 2. Materials and Methods

### 2.1 Study Participants

All subjects were participants in a longitudinal study of T2DM and its complications conducted in the Gila River Indian Community in Central Arizona between 1965 and 2007 [17]. T2DM was diagnosed per 1997 American Diabetes Association criteria [18] based on fasting and 2-hour post-load plasma glucose during a 75 g oral glucose tolerance test or during the course of routine clinical care. Since 1982, urine albumin was measured by nephelometric immunoassay [19]. Serum and urine creatinine concentrations were measured using a Technicon Autoanalyzer and ratios of urinary protein to creatinine (mg protein/g creatinine) and albumin to creatinine (mg albumin/g creatinine) were calculated. This investigation utilized 3 different study samples: a case-control study of diabetic ESRD [20], a family-based study of diabetic individuals who participated in the linkage study for nephropathy [21], and a cohort of diabetic individuals with longitudinal measures of GFR [22]. The case-control study included 107 individuals with T2DM and ESRD (defined as a need for dialysis or kidney transplantation) and 108 control subjects with T2DM duration >10 years at their last examination, and a maximum urinary albumin-to-creatinine ratio observed in the longitudinal study of < 300 mg/g [20]. All individuals were full Pima/Tohono O'odham heritage and none was a first-degree relative of another individual in the sample.

The family-based group included individuals who participated in the original genome-wide linkage study, including sibships potentially informative for association studies of diabetic nephropathy (≥ 1 sibling with diabetes, urinary protein measurements and available DNA).

This sample included 148 individuals with nephropathy and 457 without nephropathy in 257 sibships. In the family study, nephropathy was defined by a urinary protein-to-creatinine ratio ≥ 500 mg/g at the last examination, and those with urinary protein-to-creatinine ratio < 500 mg/g (including those with undetectable protein by dipstick) at their last examination were considered unaffected. This level of proteinuria reflects established kidney disease that frequently progresses to renal failure [23]. Subjects with diabetic ESRD were considered to have nephropathy, even if there were no measurements of proteinuria. Although diagnosis of diabetic nephropathy in these studies was based on clinical evidence alone, previous detailed post-mortem histological examination of the kidneys [24] and several kidney biopsy studies [25, 26] have found that diabetic glomerulosclerosis is, by far, the predominant cause of kidney disease among Pima Indians with T2DM.

The third cohort, in whom GFR was estimated by urinary clearance of iothalamate, consisted of 140 individuals with T2DM who were recruited in 4 groups (30 with newly diagnosed T2DM, 20 with T2DM for at least 5 years and normal albuminuria at baseline, 53 who had T2DM at least 5 years and microalbuminuria, and 37 who had T2DM at least 5 years and macroalbuminuria). Participants with microalbuminuria or macroalbuminuria were followed longitudinally with GFR measured every 6 months by urinary clearance of iothalamate. The remaining groups had 2 measures of GFR. The 3 different samples overlapped in that 55 individuals were included in both the family and case-control studies, but not in the GFR study; 56 were included in the family and GFR studies but not the case-control study; 19 were included in both the GFR and case-control studies but not in the family study and 16 were in all 3 studies.

Studies were approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases, the Translational Genomics Research Institute, and the Council of the Gila River Indian Community. All subjects provided written informed consent.

### 2.2 Single Nucleotide Polymorphism (SNP) selection and genotyping

SNPs tagging the entire *CNDP1* locus plus 2 kb sequence flanking either end were selected using genotype data from Chinese (CHB) individuals from the HapMap resource [27]; the CHB population was chosen as it generally provides the most accurate tags for American Indian populations [28]. In total, 19 SNPs with a minor allele frequency ≥0.10 were selected using the Tagger algorithm [29] with $r^2 > 0.8$ taken as indicative of redundancy. Markers included rs7229005, rs17089382, rs7239132, rs12605520, rs12964454, rs11876996, rs12964208, rs733686, rs7242384, rs2346064, rs12327522, rs4329999, rs11659237, rs7506957, rs8087768, rs12326826, rs12957330, rs2098910, rs7244647. We also genotyped the [CTG] microsatellite marker, *Mannheim allele (D18S880)*, *rs17818077*, and *rs11151964* based on findings of association reported in previous studies [11, 12, 30, 31]; *rs11151964* was monomorphic in this population. All SNPs except *rs11876996* were genotyped using the iPLEX® assay in conjunction with the MassARRAY® platform (Sequenom, Inc; La Jolla, CA). Primers and multiplex conditions were designed using the Assay Design v3.1 software, and DNA amplification and iPLEX primer extension were performed according to the manufacturer's protocol (Sequenom). Marker *rs11876996* could not be accommodated in a multiplex assay design and was genotyped singly by allelic discrimination PCR (AD-PCR) in conjunction with the 7000 Sequence Detection System according to the manufacturer's protocol (Applied Biosystems; Foster City, CA). The microsatellite marker *D18S880* was genotyped using standard methods [20]. The observed genotype frequency for each SNP was assessed for deviation from Hardy-Weinberg equilibrium (HWE) using a chi-square test. The distribution of genotypes did not differ significantly from HWE for any of the markers.

### 2.3 Data Analysis

Descriptive analyses of demographic data were conducted using t-tests for continuous variables and chi-square tests to compare proportions. In the case-control study, the strength of the association between genotypes and affection status was assessed by the odds ratio (OR), calculated by logistic regression. For these analyses, an additive model was used in which the genotype was coded as a numeric variable representing the number of alleles; thus, the OR shown designates the odds for ESRD associated with each copy of a designated allele. Similar tests were used in the family-based study. To account for dependence introduced by inclusion of family members, the logistic regression was conducted with a non-linear mixed model procedure that included a random effect for sibship plus fixed effects for genotype, age, sex and duration of diabetes. The strength of association of genotype with GFR was assessed using a linear mixed model including genotype, age, sex and duration of diabetes (with a quadratic term) as fixed effects. A random effect representing individual was included to account for multiple measurements of GFR in each person, assuming an autoregressive correlation structure. To correct for multiple comparisons, we estimated the effective number of variants typed by the method of Duggal et al [32]. Using the Pima genotypes, haplotype blocks were created using the solid “spine” method with D’>0.8 taken as the criterion to extend the spine; the effective number of markers was taken as the sum of the number of blocks plus the number of SNPs that fell outside of blocks [32]. Statistical analyses were conducted using Stata statistical software, version 10.0 (StataCorp LP; College Station, Texas) and SAS version 9.1 (SAS Institute; Cary, North Carolina).

### 2.4 Power Calculations

Table 1 shows the smallest odds ratio detectable with 80% at $p<0.05$ for a range of allele frequencies for the case-control and family studies. For the case control study we assume, as suggested by the cumulative incidence of ESRD and heavy proteinuria at ~20 years of diabetes duration in this population [33, 34] that cases represent the upper 15% of the liability distribution for nephropathy and that controls represent the lower 40%. For the family study we estimate by simulation that the effective sample size is 133 affected and 395 unaffected individuals for a dichotomous trait with a prevalence of 24% (i.e., the upper 24% and the lower 76% of the liability distribution). With these assumptions the minimal effect sizes were calculated using the formulae described by Hanson et al [35]. The results of these calculations, shown in Table 1, indicate that the present case-control study has sufficient power to detect an association accounting for ~1.5% of the variance in liability, which, given the selection strategy, corresponds to an odds ratio of 1.7-3.8 per copy of the risk allele, depending on its frequency. Likewise, the family study has sufficient power to detect an association accounting for ~2.7% of the variance, corresponding to an odds ratio of 1.5-2.6 per copy of the risk allele.

## 3. Results

Characteristics of participants in each of the studies are shown in Table 2. There were no differences in age of onset or duration of diabetes between cases and controls, in part, by design, because controls were selected to have long duration diabetes. In the family study, individuals who had nephropathy were older (mean ± SD, 51 ±12 years vs. 42 ±12 years) and had a longer duration of diabetes (18 ±8 years vs. 8 ±7 years) than those who did not have nephropathy. In the GFR study, the GFR measured at the first examination showed marked differences among the different groups, being higher in those with diabetes without macroalbuminuria and lowest among those with macroalbuminuria. The mean (± SD) GFR was 143 ± 39 ml/min among those with newly diagnosed diabetes, 152 ± 42 ml/min among

those with long duration diabetes and normal albuminuria, 152 ± 48 ml/min among those with microalbuminuria and 119 ± 45 ml/min among those with macroalbuminuria.

We first assessed linkage disequilibrium (LD) among the 21 markers that were genotyped in study samples. By the "spine" of linkage disequilibrium method, these markers constituted 7 haplotype blocks (Figure 1). The *CNDP1* gene is comprised of 12 exons and haplotype blocks were distributed as follows: block 1 encompasses exon 1 and part of intron 1; block 2 is contained within intron 1; block 3 contains part of intron 1 and exon 2; block 4 contains part of intron 2 and extends into intron 6, containing exons 3-6; block 5 contains part of intron 6, exon 7 and part of intron 7; block 6 contains part of intron 9, exon 10 and part of intron 10; and block 7 contains part of intron 10, exon 11 and part of intron 11. The presence of 7 haplotype blocks suggests a total of 7 independent statistical tests [32], thus, using a Bonferroni correction, we estimated the p-value necessary to achieve a locus-wide type I error of 0.05 to be $P<0.0071$ (0.05/7).

In the case-control study, ESRD was not significantly associated with any of the genotyped markers (Table 3). The Mannheim allele, which was protective against diabetic nephropathy in some studies, was observed only 3 times in 1 affected and 2 unaffected individuals.

In the family study, rs12957330 and rs17817077 were associated with diabetic nephropathy (Table 3), but following adjustment for multiple comparisons, these associations were no longer statistically significant. The Mannheim allele was observed in 18 individuals (5% of affected individuals and 3% of unaffected), all of whom were heterozygous.

In the GFR study, 3 variants in *CNDP1* were nominally associated with GFR: rs12964454, rs7244647, and rs7229005. The association with rs7229005 remained statistically significant after correction for multiple comparisons. The Mannheim allele was observed in 6 individuals, all of whom were heterozygous. On average, these individuals had a higher GFR (by 18.3 ml/min), but the difference was not statistically significant.

## 4. Discussion

In this study, we investigated *CNDP1* as a candidate gene for kidney disease in 3 different study samples comprised of Pima Indians with T2DM. We observed nominally significant associations between *CNDP1* variants and diabetic nephropathy and GFR, and these findings are consistent with a role for this locus in mediating susceptibility to diabetic kidney disease.

Prior investigations of *CNDP1* and susceptibility to diabetic kidney disease have produced results that have often been in conflict. Speculation that 18q might harbor a susceptibility gene for diabetic nephropathy followed rather strong findings of linkage in this region, in the vicinity of *CNDP1*, in 19 Turkish extended families, with subsequent confirmation in Pima Indian families who participated in the present family study [10]. Subsequently, in the multiethnic Family Investigation of Nephropathy and Diabetes (FIND), linkage for diabetic nephropathy in this region was observed in families of both European American and American Indian ancestry, but not in families of Mexican American or African American ethnicity [9]. In a separate study, Bowden et al. [8] observed linkage for ESRD in this region in African American families. More recently, a genome-wide association study in Caucasians with type 1 diabetes (T1DM) identified 18q22 as a key region for ESRD susceptibility [30]. Fine mapping studies of the initial linkage peak, reported that carriers of the 5-leucine allele (i.e., the *CNDP1* Mannheim variant) at *D18S880*, which encodes a tri-nucleotide repeat in the signal peptide sequence of exon 2, had a lower risk of developing diabetic nephropathy compared with individuals with alternative alleles [12]. Although this variant was also associated with nephropathy in an independent study of European

Americans with T2DM [11], the association was not confirmed in a large study of European Americans with T1DM [31] or in a large study of Europeans with T1DM [36]. A study in African Americans with T2DM also did not find associations with the 5-leucine allele; instead, alternative, novel haplotypes within the *CNDP1-CNDP2* locus were associated with disease in African Americans [14, 15].

It is not clear at present whether the discrepancies among studies are simply due to chance or if they reflect heterogeneous genetic effects in different populations. In the present study, the *CNDP1* "Mannheim allele" was not significantly associated with nephropathy. Its frequency was only 0.02 resulting in low power for association, and correspondingly wide confidence intervals around the odds ratio; in fact the confidence intervals are consistent with the effect reported in other populations. However, the present sample size is comparable to many other studies of *CNDP1* polymorphisms. The frequency of this putatively protective allele is lower than reported in most other populations. The frequency is ~0.60 in European and African populations, while it is somewhat lower in South Asians at ~0.46 [11, 13, 15]. The frequency is much lower in Chinese and, at 0.10, is closer to that reported in the present study [37]. Although the low frequency of the Mannheim allele in Pimas results in low power to assess its association with nephropathy in the present study, it also suggests that this allele is unlikely to account for a major fraction of the individual variation in liability to ESRD in this population. Nonetheless, it is possible that the low frequency of the putatively protective allele could account, in part, for the population's high risk of nephropathy.

In the family study, rs17817077, located in block 1 (Figure 1) was nominally associated with nephropathy. This SNP was also associated with diabetic nephropathy in European Americans with T1DM, but in the opposite direction (odds ratio=1.3 per copy of the G allele) [30]. It is not clear why these results are discordant, but one possible reason may be due to disparate linkage disequilibrium patterns such that the functional variant(s) occur on different haplotypes in different populations (i.e., the "*flip-flop*" effect) [38]. Such inconsistent associations may also occur by chance, however, and further replication studies with detailed haplotyping across populations are needed to distinguish these possibilities. Neither the association with rs1780177 nor rs12957330, which was also nominally associated with nephropathy in the family study, was strong enough to explain the linkage in this region in this population (the p-values for a significant reduction in the linkage were 0.57 and 0.83, respectively, as assessed by an extension of the Haseman-Elston method [39]).

Although the findings reported here are consistent with a role for *CNDP1* variants in diabetic nephropathy in Pima Indians, there are some issues that may limit the interpretation of the results. First, sample sizes were small, and they have adequate power to detect only moderate to large effects. As shown in our detailed power calculations, we estimate that the smallest odds ratio detectable at $p<0.05$ with 80% power for a risk allele with frequency 0.2 is 1.99 for the case control study or 1.65 for the family study) [35]. Given linkage in the family study and selection of individuals discordant for susceptibility to nephropathy in the case-control study, one might expect larger odds ratios than in a study of randomly selected individuals. Additionally, replication studies need to be performed in comparable populations with similar design and disease definitions.

In addition, our SNP selection strategy utilized the HapMap resource and genotype data from Han Chinese individuals. Although tag SNPs derived from the East Asian HapMap populations generally capture most of the surrounding variation in American Indians [28], this approach would miss markers with effects on disease susceptibility that are specific to the Pima population. It is not possible to determine how well the present set of SNPs tagged

SNPs that are not represented in HapMap. For example, McDonough, et al. identified risk haplotypes in the *CNDP1* SNP rs4892247 and rs6566810 (which is in the nearby *CNDP2*) that were associated with diabetic ESRD in African Americans [15]; but these SNPs were not in the HapMap database. Additionally, because of the power available with our study samples, we limited selection of tags to SNPs with minor allele frequency ≥0.10, and thus, rare variants would not be included in this study. For example, rs11151964, which was associated with diabetic ESRD in European Americans with T1DM [30], was not selected as a tag because the frequency of the minor allele is only 0.056. Because of its previously reported association, this marker was genotyped but was monomorphic for the G allele, which was the low risk allele in Caucasians with T1DM.

Further, we acknowledge that proteinuria or ESRD may not be optimal variables for classifying diabetic kidney disease, since GFR and structural changes are often detected independently of heavy proteinuria. Thus, we included GFR as a physiologically relevant phenotype. The strongest association (p=0.001) was with marker rs7229005, which remained statistically significant after correction for the effective number of variants typed in the present study. However, the appropriate correction for multiple comparisons in genetic studies remains controversial. This marker has not been associated with diabetic kidney disease in previous studies, and many genetic statisticians suggest that the stringent p-values for genome-wide significance (e.g., $p<7 \times 10^{-8}$) should be used for associations without strong prior evidence for association. Thus, the findings here, while preliminary, suggest that this marker be investigated in other populations.

These limitations notwithstanding, *CNDP1* is a good biological candidate for diabetic nephropathy. The carnosine pathway plays a critical role in glucose metabolism. Non-transgenic *db/db* mice supplemented with L-carnosine have significantly lower fasting plasma glucose (FPG) levels and a delayed diabetes onset compared with untreated *db/db* mice, while transgenic *db/db* mice expressing the human carnosine dipeptidase 1 variant (hCN1) developed hyperglycemia earlier and had significantly higher FPG and HbA1c compared with untreated control mice [40]. Carnosine inhibits *in vitro* production of fibronectin, collagen type VI, and TGF-beta in renal cells under hyperglycemic conditions [12]. Together these findings underscore the impact of carnosine levels in response to hyperglycemia.

In summary, *CNDP1* may play a role in diabetic kidney disease in Pima Indians. This population has a very high prevalence of T2DM and rates of progression, diabetic nephropathy and ESRD. We did not identify common variants in or near *CNDP1* with strong associations that might explain the linkage of diabetic nephropathy on 18q22.3. However, the association between rs7229005 and GFR might implicate this gene in susceptibility to diabetic nephropathy. This possibility warrants investigation in other populations.

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## Abbreviations

**CNDP1** carnosine peptidase 1 gene

| | |
|---|---|
| **ESRD** | end-stage renal disease |
| **FPG** | fasting plasma glucose |
| **GFR** | glomerular filtration rates |
| **HWE** | Hardy-Weinberg equilibrium |
| **MAF** | minor allele frequency |
| **NCRR** | National Center for Research Resources |
| **NIH** | National Institutes of Health |
| **OR** | odds ratio |
| **SNP** | single nucleotide polymorphism |
| **T1DM** | type 1 diabetes mellitus |
| **T2DM** | type 2 diabetes mellitus |

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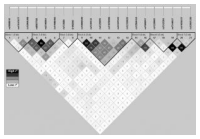

**Fig 1.**

Pairwise linkage disequilibrium between *CNDPI* markers in 388 individuals from the study cohort (selecting one individual per family). Linkage disequilibrium is shown in terms of $r^2$ which is measure of concordance between markers. Haplotype blocks were constructed by the solid "spine" method with D' > 0.8 as the criterion to extend the "spine".

## Table 1

Minimal detectable effect size at P<0.05 with 80% power for case-control and family association studies.

| | Case-Control Study | | Family Study | |
|---|---|---|---|---|
| MAF | OR | $h^2$ | OR | $h^2$ |
| 0.05 | 3.80 | 0.017 | 2.57 | 0.030 |
| 0.10 | 2.54 | 0.016 | 1.96 | 0.029 |
| 0.20 | 1.99 | 0.015 | 1.65 | 0.028 |
| 0.30 | 1.82 | 0.015 | 1.55 | 0.027 |
| 0.40 | 1.75 | 0.015 | 1.50 | 0.027 |
| 0.50 | 1.73 | 0.015 | 1.49 | 0.027 |

OR represents the odds ratio per copy of the risk allele and $h^2$ is the proportion of variance in liability to disease explained by the variant.

**Table 2**

Descriptive analyses of subjects in each study

| | **Case Control Study** | | **Family Study** | | **GFR Study** | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Cases (N=107) | Controls (N=108) | Presence of Nephropathy (N=148) | Absence of Nephropathy (N=457) | Newly diagnosed T2DM (N=30) | T2DM ≥ 5 years with normal albuminuria (N=20) | T2DM ≥ 5 years with microalbuminuria (N=53) | T2DM ≥ 5 years with macroalbuminuria (N=37) |
| Age (mean ± SD), years | 36 ± 9 [a] | 38 ± 10 [a] | 51 ± 12 | 42 ± 12 | 36 ± 11 | 44 ± 10 | 43 ± 9 | 48 ± 12 |
| Female gender, % | 56 | 68 | 38 | 37 | 57 | 60 | 62 | 47 |
| Mean duration of disease (mean ± SD), years | 21 ± 5 | 20 ± 7 | 18 ± 8 | 8 ± 7 | 0.9 ± 1.0 | 13.8 ± 5.4 | 13.0 ± 5.1 | 16.5 ± 9.8 |

[a] Represents age at onset of diabetes

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**Table 3**

Relationship of SNPs with measures of nephropathy and kidney function

| Locus | Chromosome Position | Allele [a] | Frequency of allele | Case Control Study [b] | | Family Study [c] | | GFR Study [e] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | OR (95% CI) | p value | Multivariate OR (95% CI) [d] | p value | beta coefficient [d] (95 % CI) | p value |
| rs2098910 | 70351582 | **G**/C | 0.77 | 1.45 (0.88, 2.39) | 0.14 | 1.61 (0.98, 2.62) | 0.06 | −3.7 (−12.9, 5.6) | 0.44 |
| **rs17817077** | 70360523 | **G**/A | 0.92 | 0.81 (0.39, 1.67) | 0.57 | **0.46 (0.21, 0.99)** | **0.05** | −5.3 (−26.5, 15.9) | 0.62 |
| rs12964208 | 70361170 | **G**/A | 0.85 | 0.89 (0.53, 1.49) | 0.66 | 0.72 (0.46, 1.13) | 0.15 | −6.5 (−17.4, 4.3) | 0.24 |
| rs8087768 | 70364052 | **T**/G | 0.80 | 0.97 (0.61, 1.56) | 0.91 | 0.74 (0.46, 1.19) | 0.22 | −5.0 (−13.9, 3.9) | 0.27 |
| rs2346064 | 70364396 | **T**/C | 0.78 | 1.03 (0.64, 1.65) | 0.91 | 0.73 (0.49, 1.07) | 0.11 | −3.1 (−11.9, 5.8) | 0.50 |
| rs17089382 | 70369681 | **C**/T | 0.86 | 1.30 (0.75, 2.24) | 0.35 | 0.91 (0.58, 1.42) | 0.68 | −2.8 (−12.9, 7.3) | 0.59 |
| rs733686 | 70374195 | **C**/T | 0.87 | 1.04 (0.59, 1.86) | 0.88 | 1.36 (0.69, 2.69) | 0.37 | −1.4 (−12.9, 10.1) | 0.81 |
| D18S880 (Mannheim) | | **6**/5 | 0.98 | 2.10 (0.30, 14.6) | 0.45 | 0.62 (0.13, 2.91) | 0.55 | −18.3 (−45.7, 9.2) | 0.19 |
| rs12605520 | 70377484 | **C**/T | 0.64 | 0.92 (0.61, 1.39) | 0.69 | 0.88 (0.63, 1.22) | 0.43 | 4.9 (−3.1, 12.9) | 0.23 |
| rs7239132 | 70377789 | **A**/C | 0.63 | 0.90 (0.60, 1.36) | 0.62 | 0.75 (0.48, 1.16) | 0.20 | 3.0 (−5.0, 10.9) | 0.46 |
| rs4329999 | 70379249 | **A**/G | 0.62 | 1.02 (0.68, 1.54) | 0.92 | 0.79 (0.56, 1.10) | 0.16 | 4.9 (−3.7, 13.6) | 0.27 |
| rs11876996 | 70382460 | **T**/C | 0.56 | 1.10 (0.75, 1.61) | 0.63 | 0.87 (0.63, 1.20) | 0.39 | −5.7 (−13.4, 2.0) | 0.15 |
| rs12327522 | 70387399 | **A**/T | 0.51 | 0.92 (0.62, 1.36) | 0.68 | 1.28 (0.92, 1.77) | 0.15 | −7.5 (−15.0, 0.0) | 0.05 |
| rs12326826 | 70387463 | **T**/C | 0.64 | 1.04 (0.66, 1.64) | 0.87 | 0.90 (0.61, 1.35) | 0.61 | −5.1 (−13.4, 3.2) | 0.23 |
| **rs12964454** | 70389710 | **G**/T | 0.61 | 1.15 (0.77, 1.71) | 0.49 | 1.17 (0.83, 1.64) | 0.36 | **−8.5 (−16.4, −0.6)** | **0.04** |
| rs7506957 | 70392315 | **G**/T | 0.84 | 1.11 (0.68, 1.820 | 0.66 | 1.42 (0.85, 2.38) | 0.19 | −6.6 (−17.0, 3.8) | 0.21 |
| **rs7229005** | 70397061 | **T**/C | 0.67 | 1.31 (0.87, 1.97) | 0.19 | 1.17 (0.82, 1.66) | 0.39 | **−13.4 (−21.3, −5.5)** | **0.001** |
| rs7242384 | 70399230 | **G**/A | 0.80 | 0.90 (0.54, 1.51) | 0.69 | 0.95 (0.59, 1.55) | 0.85 | 1.8 (−7.4, 11.1) | 0.70 |
| **rs7244647** | 70399798 | **C**/T | 0.96 | 0.48 (0.20, 1.03) | 0.09 | 0.41 (0.14, 0.17) | 0.09 | **18.8 (1.4, 36.1)** | **0.03** |
| rs11659237 | 70401742 | **C**/T | 0.81 | 0.95 (0.56, 1.60) | 0.85 | 0.97 (0.65, 1.43) | 0.87 | 2.3 (−7.1, 11.8) | 0.63 |
| **rs12957330** | 70402360 | **G**/A | 0.97 | 0.74 (0.24, 2.25) | 0.60 | **0.29 (0.08, 0.98)** | **0.04** | 10.1 (−21.8, 42.1) | 0.53 |

[a] Underlined and bold allele is considered the denoted allele for which frequencies are given (odds ratios and regression coefficients are also given per copy of this allele).

[b] Association of *CNDP1* markers with ESRD (case-control group)

[c] Association of *CNDP1* markers with diabetic nephropathy in family based study group

[d] Adjusted for age, sex, and duration of diabetes

[e] Association of *CNDP1* markers with glomerular filtration rate