

## Commentary

### The (CTG)<sub>n</sub> repeat polymorphism in *CNDP1* gene: new insights into an old molecule

Diabetic nephropathy (DN) is a chronic complication of diabetes mellitus clinically characterized by persistent albuminuria and reduced kidney function<sup>1</sup>. It affects 20-30 per cent of diabetic patients and South Asians with diabetes have a higher prevalence and faster progression of nephropathy than Caucasians<sup>2</sup>. Diabetic nephropathy is a leading cause of end-stage renal disease and is associated with increased cardiovascular mortality<sup>1</sup>. Although the poor glycaemic control, longer duration of diabetes, and hypertension are among the main risk factors for DN, clinical, familial, and population-based studies showed that genetic factors play a role in the development and progression of this complication<sup>1,3</sup>.

Linkage analysis and candidate gene approach have identified several potential chromosomal regions and genes involved in the pathogenesis of DN. However, positive findings were replicated for only a few of them in subsequent studies<sup>3,4</sup>. Genome-wide association studies are beginning to identify genetic risk factors for DN across the entire genome. However, as pointed by Kwak and Park<sup>3</sup>, most of these have also been underpowered and heterogeneous in relation to study design, inclusion criteria, and phenotype definition. In this issue, Yadav *et al*<sup>5</sup> report the association of a trinucleotide (CTG)<sub>n</sub> repeat polymorphism (D18S880) in the coding region of carnosinase-1 gene (*CNDP1*) with DN in a north Indian population.

Carnosine is an anti-ageing dipeptide that has antiproliferative properties and antioxidant activity<sup>6</sup>. It chelates metal ion, has *pH*-buffering activity, scavenges reactive oxygen species and peroxy radicals, inhibits protein carbonylation, prevents the formation of protein cross-links, advanced glycation and lipoxidation end products, reverses protein glycation, protects telomeres from damage by reducing its shortening rate, and improves enzymatic and non-

enzymatic antioxidant activity<sup>6,7</sup>. Carnosine regulates calcium release and sensitivity in skeletal muscle cells, potentiates cardiac contractility, and reduces ischaemia/reperfusion damage in different animal models and human organs, including the kidney. Moreover, carnosine has systemic vasodilator effect, acts as a natural inhibitor of the angiotensin converting enzyme (ACE), decreases blood pressure<sup>6</sup>, restores erythrocyte deformability, and reduces the synthesis of matrix proteins in podocytes and mesangial cells<sup>7</sup>.

Carnosine is synthesized by carnosine synthase and readily hydrolyzed by carnosinase into  $\beta$ -alanine and L-histidine. Carnosinase exists in two differentially expressed isoforms (CN1 and CN2) that are encoded by different genes (*CNDP1* and *CNDP2*) located side by side on human chromosome 18q22.3. Serum carnosinase (CN1) is a selective metallopeptidase secreted by the liver into the circulation and found in serum and brain, whereas cytosolic carnosinase (CN2) is non-specific and ubiquitously expressed (but not in serum). Carnosinase-1 is undetectable in newborns and is increasingly expressed with age<sup>6,7</sup>, attaining higher levels in women than men. Activity of carnosinase-1 exhibits large interindividual variability, but limited intraindividual variation<sup>6</sup>.

In transgenic mice, the overexpression of carnosinase-1 increases blood glucose and reduces the insulin levels<sup>7</sup>. On the other hand, administration of carnosine orally reduces blood glucose, increases serum insulin levels, and reduces the oxidative damage, by preserving or increasing pancreatic beta-cell mass<sup>6,7</sup>. In diabetic mouse model, renal carnosinase-1 activity is increased and treatment with carnosine normalizes its activity, decreases proteinuria and renal vascular permeability<sup>7</sup>, reduces the expression of pro-apoptotic proteins, restrains glomerular apoptosis, and prevents podocyte loss<sup>6</sup>.

Based on cultured renal cells obtained from healthy donors and biopsy tissue from patients with type 2 diabetes, Peters *et al*<sup>8</sup> have proposed that the human kidney has an intrinsic capacity to metabolize carnosine. Carnosinase-1 was located primarily in distal tubules and had higher expression and activity levels in tubular cells and podocytes. In diabetes patients with renal damage, carnosinase-1 was reallocated from distal to proximal tubules. In the proximal tubules, the levels of carnosinase-1 were higher in patients with DN as compared to healthy controls. Janssen *et al*<sup>9</sup> showed that the carnosinase-1 expression was increased in the kidneys of patients with DN, especially in podocytes.

Janssen *et al*<sup>9</sup> were the first to identify the trinucleotide repeat in exon 2 of the *CNDPI* gene in Europeans and Arabs and to report an association between this genetic variant and DN. The CTG repeat codes for a leucine repeat in the signal peptide of the carnosinase-1 precursor, and the number of leucine residues determines the efficiency of the protein secretion, thereby altering the concentration of this enzyme in the circulation<sup>9</sup>. The number of repeats varies from 4 to 8 (4L-8L), and the 5L and 6L are the most frequent alleles. Functional assays demonstrated that carnosinase-1 secretion is significantly higher in cells expressing variants with more than five leucines in the signal peptide. Hence, this would explain why diabetic patients homozygous for the 5L allele are less susceptible to DN and have lower serum carnosinase activity<sup>6</sup>. Some studies have investigated the association of the (CTG)<sub>n</sub> repeat polymorphism with DN in both type 1 and type 2 diabetes, resulting in seemingly conflicting findings. This led two research groups to conduct a meta-analysis, which confirmed that this polymorphism was associated with DN susceptibility in type 2 diabetes, but not in type 1 diabetes<sup>10,11</sup>. Recently, a cohort study in Caucasians with type 2 diabetes from Netherlands did not observe differential progression of renal function loss according to the (CTG)<sub>n</sub> repeat polymorphism. However, at baseline the glomerular filtration rate was higher among subjects homozygous for the 5L allele than in subjects with other genotypes<sup>12</sup>.

In line with this, Yadav *et al*<sup>5</sup> enrolled and genotyped more than 500 individuals for the D18S880 microsatellite. The frequency of 5L-5L genotype was lower in patients with DN compared to those without this complication and healthy controls. They also found that homozygosity for the 5L allele was independently associated with reduced risk of DN. Despite the inherent limitations of the case-control studies and the lack of data on levels of carnosinase-1 in South Asians,

the study conducted by Yadav *et al*<sup>5</sup> supports the assumption that patients with type 2 diabetes who carry the 5L-5L genotype are less susceptible to have DN. In their meta-analysis, Zhu *et al*<sup>11</sup> also observed that such association is especially valid for the Caucasian population. In fact, no association between the (CTG)<sub>n</sub> repeat polymorphism and DN was detected in African Americans<sup>13</sup>, Mexican Americans<sup>14</sup>, American Indians<sup>15</sup>, and Japanese<sup>16</sup> with type 2 diabetes. In this context, the study reported by Yadav *et al*<sup>5</sup> is the first to investigate the association of this polymorphism with DN in South Asians and brings new evidence suggesting that not only Caucasians homozygous for the 5L-5L genotype may be protected from this complication. Taken together, these findings reinforce the proposal that diabetes patients homozygous for the 5L allele of *CNDPI* gene are less prone to develop DN, because low carnosinase secretion and activity promotes less carnosine hydrolysis, and high circulating carnosine levels act as a protective factor against adverse effects of hyperglycaemia on renal cells<sup>6,7</sup>.

Although other variants have been described in *CNDPI* and *CNDP2* genes and are reported to be associated with DN<sup>13,15-18</sup>, accumulating evidence from experimental and clinical studies suggest that the (CTG)<sub>n</sub> repeat polymorphism, alone or in linkage disequilibrium with other variants in the same locus, might be one of the causative variants of DN. Genome linkage scans for DN in type 2 diabetes in multiethnic populations have mapped susceptibility loci to chromosome 18q22.3-23, a region that also includes the *CNDPI* and *CNDP2* genes<sup>4,6</sup>. A recent genome-wide association study in African Americans also observed a nominal sign for *CNDPI* gene<sup>18</sup>. Inconsistent findings from genetic association studies have been attributed to the ethnicity<sup>7,11</sup>, linkage with other risk variants<sup>7</sup>, small sample size, inadequate statistical power<sup>4,11</sup>, confounding by population stratification, different phenotype definitions, influence of environmental factors, contribution of rare variants<sup>4</sup>, type of diabetes, publication bias<sup>11</sup>, study design, and duration of diabetes. In spite of all these factors, the *CNDPI* gene remains as a promising candidate gene for susceptibility to DN among the several loci that have already been identified.

As pointed by Boldyrev *et al*<sup>6</sup>, most of the research on the therapeutic potential of carnosine is conducted in animal models of human diseases, mainly in rodents, in which treatment with carnosine improved vision and neurological functions. Apart from its application to sport and nutrition science, it would be

worth to study whether treatment with carnosine could prevent or ameliorate DN in humans. Considering that approximately 40 per cent of the population are homozygous for the 5L allele of (CTG)<sub>n</sub> repeat polymorphism in *CNDP1* gene and seem to be less prone to develop DN, it is needed to evaluate whether the majority of diabetic patients (who do not have the 5L-5L genotype) would be benefited from supplementation with carnosine or with a more intensive monitoring. Carnosine is proposed to exert its protective effects against the development of DN due to its antioxidant capacity. However, as carnosine inhibits ACE, it may also protect against renal damage by reducing blood pressure. Thus, it would be interesting to investigate whether the (CTG)<sub>n</sub> repeat polymorphism in *CNDP1* gene interacts with ACE inhibitors widely used by diabetic patients, contributing to prevent renal damage.

Diabetes mellitus is a serious public health problem. It is estimated that India will have nearly 70 million individuals with diabetes by 2025. This will result in a huge burden of complications, which in turn, affects the economy in the order of billion dollars yearly with both direct and indirect costs<sup>2</sup>. More efforts to unravel the molecular and cellular bases of diabetic complications are needed to improve their prevention and treatment. Understanding how gene variants affect carnosine metabolism may help in the development of clinical approaches for identifying the patients who are more susceptible to DN and for treating those who have low levels of carnosine. This is an area still unexplored, and certainly, carnosine-carnosinase makes part of an intriguing homeostatic system.

**Kátia G. Santos**

Laboratory of Human Molecular Genetics,  
Lutheran University of Brazil (ULBRA)  
Canoas, RS, Brazil  
kgsantos2010@gmail.com

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