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High Plasma Pentraxin 3 Levels in Diabetic Polyneuropathy Patients with Nociceptive Pain

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Diabetic polyneuropathy is the most common neurologic complication of diabetes mellitus. Underlying mechanisms of diabetic polyneuropathy are related to various metabolic and inflammatory pathways. Pentraxin 3 (PTX3) is an acute phase protein that is produced locally at the inflammatory sites by several cell types. Thioredoxin binding protein 2 (TBP2) is a thioredoxin regulator involved in intracellular energy pathways and cell growth. We measured the plasma levels of PTX3 and TBP2 in type 2 diabetic patients (n = 27) with pain complaints and compared their levels with those of healthy age- and sex-matched subjects (n = 24). Moreover, the diabetic patients were divided into two groups using the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) pain scale: patients with nociceptive pain that is caused by tissue damage and patients with neuropathic pain that is caused by nerve damage. Patients with LANSS scores of < 12 were considered to have nocicceptive pain (n = 15), while patients with LANSS scores of ≥ 12 were considered to have neuropathic pain (n = 12). We found that PTX3 levels were significantly higher in diabetic patients compared to controls ($p = 0.03$), but there was no significant difference in the TBP2 levels. Importantly, patients with nociceptive pain had significantly higher PTX3 levels compared to patients with neuropathic pain ($p < 0.05$). Thus, plasma PTX3 levels can be helpful for discrimination of nociceptive pain from neuropathic pain in diabetic patients. We propose that PTX3 may contribute to the onset of nociceptive pain.

Keywords: diabetes mellitus; diabetic polyneuropathy; neuropathic pain; nociceptive pain; pentraxin 3
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Introduction

Diabetes mellitus (DM) is a worldwide epidemic disease in developed countries. The major neurologic complication of DM is neuropathy, and the lifetime incidence of diabetic polyneuropathy in patients with type 2 DM is approximately 50% (Dyck et al. 1993, 1995; Edwards et al. 2008). The most common type of diabetic polyneuropathy is distal symmetrical polyneuropathy, and it is predominantly axonal, involving longest myelinated and unmyelinated sensory axons (Russell and Zilliox 2014). Diabetic peripheral neuropathic pain occurs in 7.5% to 24% of all patients with DM with or without diabetic polyneuropathy and can be one of the most painful complications leading to decrease in life quality of DM patients (Zilliox and Russell 2011). Pain is divided into nociceptive or non-neuropathic and neuropathic pain. Nociceptive pain results from actual tissue damage or potentially tissue-damaging stimuli with normal neural signaling to the brain, whereas neuropathic pain arises as a direct consequence of a lesion or a disease affecting the somatosensory system, leading to peripheral or central sensitization, related to damage of inhibitory functions of the nervous system (Basic-Kes et al. 2009). However, not all patients with diabetes have peripheral neuropathy caused by diabetes.

DM can predominantly or entirely affect small myelinated (Aδ) fibers or unmyelinated C fibers, relatively sparing large myelinated fibers, and small-nerve-fiber involvement may be the earliest detectable sign of the neuropathy (Sumner et al. 2003). The specific fiber types involving this process include both small somatic and autonomic fibers and cause neuropathic pain and autonomic dysregulation. Assessment of only large fiber function in DM patients is the main limitation in conventional nerve conduction stud-

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ies. Firstly, diagnosis of small fiber neuropathy is determined by history and physical exams, and then functional neurophysiological testing and skin biopsy evaluation can provide diagnostic confirmation of the disease (Hovaguimian and Gibbons 2011).

The underlying mechanism of diabetic polyneuropathy is extremely complex and likely related to various metabolic and inflammatory pathways inducing individually or superimposed on ischemic nerve lesions (Said 2007). However, it is difficult to determine the mechanism because any factor leading to cellular stress may induce an inflammatory response or inflammation that leads to polyneuropathy (Hotamisligil and Erbay 2008).

The extension of a systemic inflammatory reaction in DM is an important reason of microvascular complications. It has been suggested that type 2 DM is an inflammatory disease (Donath and Shoelson 2011). Prospective and cross-sectional studies showed that type 2 DM was associated with the increases of acute-phase proteins such as C-reactive protein (CRP), fibrinogen, plasminogen activator inhibitor, cytokines and chemokines (Pickup et al. 1997; Spranger et al. 2003; Herder et al. 2005, 2009).

Pentraxin 3 (PTX3) is a component of the humoral arm of innate immunity and belongs, together with the CRP and the other acute phase proteins, to the pentraxins superfamily (Bonacina et al. 2013). Pentraxins are a superfamily of soluble, multifunctional, pattern recognition proteins (Garlanda et al. 2005). Pentraxins are structurally divided into two groups: short pentraxins and long pentraxins. CRP and amyloid P-component are classic short pentraxins produced by the liver, whereas PTX3 is a prototype for a long form, produced locally at the inflammatory sites by several cell types, primarily mononuclear phagocytes, fibroblasts, dendritic cells, smooth muscle cells and endothelial cells in response to inflammatory signals (Mantovani et al. 2008). PTX3 deficiency is associated with increased inflammation in vascular diseases. On the other hand, its overexpression limits carotid restenosis after angioplasty. These observations imply that PTX3 has a cardiovascular protective effect, associated with the ability to tune inflammatory responses (Bonacina et al. 2013). PTX3 is positively associated with development and progression of diabetic retinopathy (Yang et al. 2014). These data suggest the role of PTX3 as a biomarker for vascular diseases.

Thioredoxin binding protein 2 (TBP2) is a multifunctional regulator involved in variety of pathways, including protein reducing activity, cancer suppression, vascular function, regulation of immunity and inflammation and metabolic diseases like DM (Masutani et al. 2012). TBP2 increases the production of reactive oxygen species, and oxidative stress, resulting in cellular apoptosis (Zhou and Chng 2013) Increased TBP2 expression causes impairment of insulin sensitivity and glucose-induced insulin secretion (Oka et al. 2009).

In this study, we aimed to measure the plasma levels of PTX3 and TBP2 in patients with diabetic polyneuropathy and to compare their results with the healthy subjects.

Methods

Study design

Twenty-seven consecutive type 2 diabetic patients with symmetrical pain and/or numbness in the feet were included in this study. All patients fulfilled ADA (American Diabetes Association) criteria for the diagnosis of DM. The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) Pain Scale was measured for the discrimination of neuropathic pain using Bennet's cut-off of 12 points (Bennett 2001). Patients with LANSS scores of < 12 were considered to have nociceptive pain ($n = 15$), and patients with LANSS scores of ≥ 12 were considered to have neuropathic pain. According to the neurological and electrophysiological examination, patients with diabetic retinopathy, diabetic nephropathy, radiculopathy, mononeuropathy, plexopathy or possible other systemic inflammatory disease, toxic or drug related causes of neuropathy or neuropathic pain were excluded from the study.

All patients underwent electrophysiological investigation. An investigator who performed the electrophysiological study was blinded to the clinical history. These tests were performed in the neurophysiology laboratories of the Umraniye Education and Research Hospital and NPIstanbul Neuropsychiatry Hospital using an electromyography machine (Medelec Synergy Electromyography Machine; Oxford Instruments, Oxford, UK). Routine motor and sensory nerve conduction studies were performed as suggested by Stalberg et al. (1999). In all patients, bilateral posterior tibial, common peroneal motor nerves and bilateral sural and superficial peroneal sensory nerves with left median, ulnar motor and sensory nerves were studied. Control group consist of 24 healthy age- and sex-matched subjects who had no risk factors for neuropathy or neuropathic pain. Blood samples were taken in the morning between 7:00 and 9:00 from the patients and control subjects. The study protocol was in accordance with the Helsinki declaration of human rights and was approved by the Uskudar University Clinical Research Ethics Committee and all patients and controls gave written informed consent to participate in the study.

Determination of plasma levels of pentraxin 3 and thioredoxin binding protein 2

Blood samples were drawn in tubes containing EDTA. The samples were centrifuged at 3,000 rpm for 10 minutes +4°C and stored at −80°C. Plasma samples of subjects were thawed and analyzed for PTX3 and TBP2 concentrations as ng/ml by using a commercially available (Boster) enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's manual. All samples were assayed on a duplicate. The O.D. absorbance was measured at 450 nm with Multiskan™ GO Microplate. Hemoglobin A1c (HbA1c) values were obtained by high-performance liquid chromatography.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software for Windows, version 11.5 (SPSS, Inc., Chicago, Illinois). Results were expressed as the mean ± standard deviation (SD). Statistical differences were assessed by Student's unpaired *t*-test, with $p < 0.05$ as a statistical significancy cut-off.

Results

The age ranges of patients and control subjects were 54.59 ± 8.98 and 54.33 ± 12.19 years, respectively (Table 1). There was no statistically significant difference between two groups according to sex (Table 1), height and body weight (data not shown). Demographic and electrophysiologic data of patients are shown in Table 2. All subjects in the study completed LANSS pain scale questionnaire, and the overall LANSS scores for the patients and control subjects (mean ± SD) were 10.67 ± 5.46 and 1.35 ± 0.71, respectively. According to the LANSS questionnaire, patients were divided into two groups. Twelve patients had neuropathic pain with the LANSS scores of 15.17 ± 3.97, and 15 patients had nociceptive pain according to the LANSS scores below 12 (7.38 ± 3.54). There was no statistically significant difference between two groups according to age, duration of DM and HbA1c. According to the nerve conduction studies, distal symmetric polyneuropathy was detected in overall 9/27 patients (33.3%), sensory axonal polyneuropathy in 5/27 patients (18.5%) and sensorimotor axonal polyneuropathy in 4/27 (14.8%).

Plasma levels of PTX3 and TBP2 were compared between patients and controls. It was found that PTX3 levels were significantly higher in diabetic polyneuropathy patients ($p = 0.03$) (Fig. 1). However, there was no significant difference between the study groups in the levels of TBP2 (Fig. 2). The PTX3 levels and TBP2 levels of patients and controls are shown in Fig. 3. There is no significant correlation between PTX3 levels and TBP2 levels for all subjects. Likewise, there is no significant correlation between PTX3 and TBP2 levels in patients with neuropathic and those with nociceptive pain (Fig. 4).

To explore whether there is a possible role of PTX3 in the onset of pain, we compared plasma PTX3 levels of patients with neuropathic and those with nociceptive pain (Fig. 5). The PTX3 levels were significantly higher in patients with nociceptive pain than those in the patients with neuropathic pain ($p = 0.04$) and controls ($p = 0.006$). Importantly, patients with neuropathic pain had the PTX3 levels similar to those of controls ($p = 0.34$) (Fig. 5).

Discussion

Neuropathic pain is defined as "pain initiated or caused by a primary lesion or dysfunction of the nervous system" and as 'burning', 'electric', 'tingling', and 'shooting' in nature (Merksey et al. 1986). While nociceptive pain is caused by stimulation of peripheral A-delta and C-polymodal pain receptors via algogenic substances, neuropathic pain is produced by damage or pathologic changes in the peripheral or central nervous systems. C-fiber induced nociceptive pain can be also a neuropathic pain, if central sensitization develops after somatic tissue injury (MacFarlane et al. 1997). Therefore, this situation can lead to diagnostic confusion, especially in chronic pain patients, with or without neuropathic component.

The primary symptoms of neuropathic pain are chronic allodynia and hyperalgesia. Allodynia is a pain responding

Table 1. The demographic data of patients with diabetic polyneuropathy and control subjects.

	Patients	Control
Numbers of Subjects (male/female)	27 (7/20)	24 (10/14)
Age of Subjects (mean ± SD)	54.59 ± 8.98	54.33 ± 12.19

Table 2. The demographic and electrophysiologic data of patients diabetic polyneuropathy.

	Number of patients (male/female)	Age (mean ± SD)	Duration of DM (mean ± SD) [Years]	HbA1c (mean ± SD) [%]	LANSS (mean ± SD)	Electrophysiologic data	
						Distal Sensory Axonal Neuropathy (number)	Distal Sensorymotor Axonal Neuropathy (number)
Patients with Neuropathic Pain	12 (2/10)	56.08 ± 10.14	8.66 ± 6.95	10.05 ± 3.66	15.16 ± 3.97	3	3
Patients with Nociceptive Pain	15 (5/10)	53.40 ± 8.09	7.00 ± 4.29	8.33 ± 2.23	7.06 ± 3.43	2	1

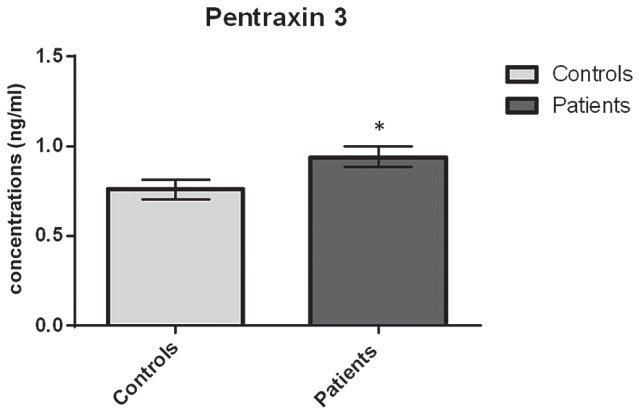

Fig. 1. Plasma pentraxin 3 levels in patients with diabetic polyneuropathy and controls.
The plasma pentraxin 3 levels were significantly higher in patients (n = 27) than those in controls (n = 22).
*p = 0.03.

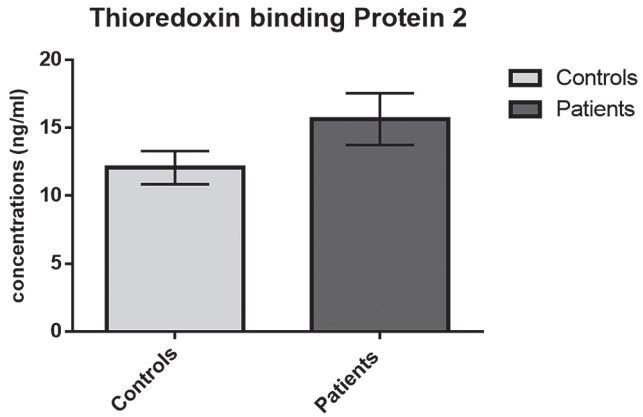

Fig. 2. Plasma thioredoxin binding protein 2 levels in patients with diabetic polyneuropathy and controls.
There was no significant difference in the plasma thioredoxin binding protein 2 levels between patients (n = 27) and controls (n = 22).

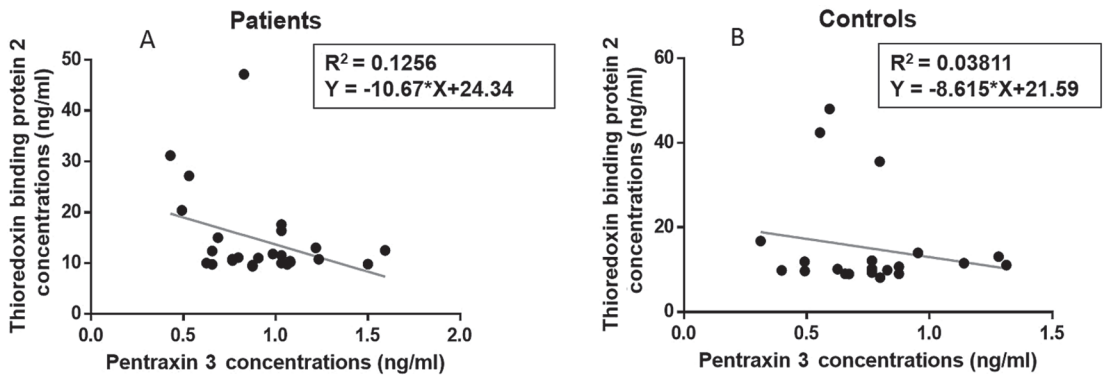

Fig. 3. No correlation between pentraxin 3 and thioredoxin binding protein 2 levels in patients and control subjects.
There was no significant correlation between pentraxin 3 levels and thioredoxin binding protein 2 levels for patients (A, n = 27) and control subjects (B, n = 22).

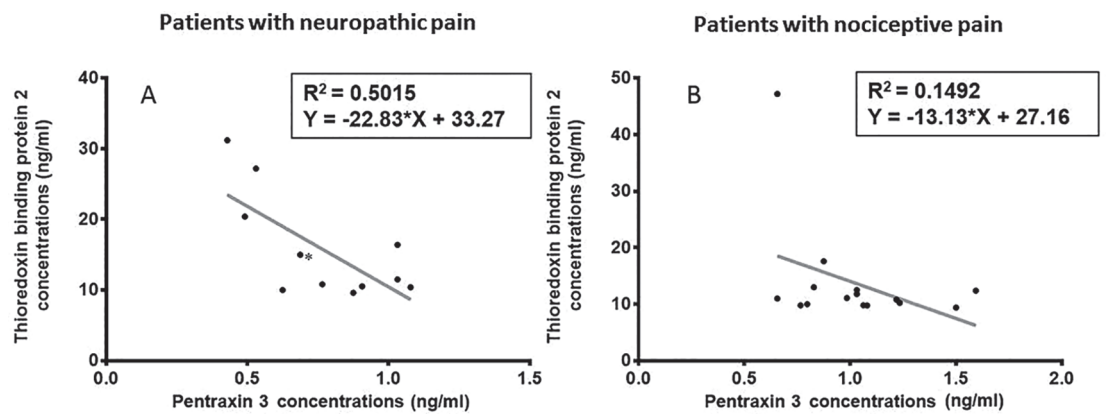

Fig. 4. No correlation between pentraxin 3 and thioredoxin binding protein 2 levels in patients with neuropathic pain and with nociceptive pain.
There is no significant correlation between pentraxin 3 levels and thioredoxin binding protein 2 levels in patients with neuropathic pain (A, n = 12) and in patients with nociceptive pain (B, n = 15).
*Two dots are completely overlapping.

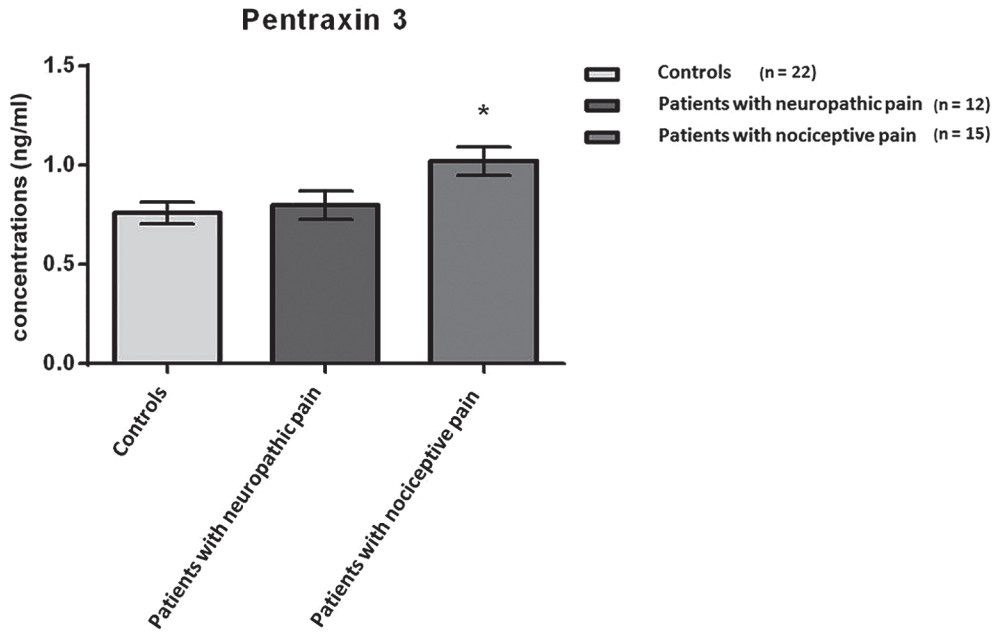

Fig. 5. Higher pentraxin 3 levels in patients with nociceptive pain.
The plasma pentraxin 3 levels were compared among controls (n = 22), patients with neuropathic pain (n = 12) and nociceptive pain (n = 15). *$p < 0.05$.

to a nonpainful stimulus (e.g., light touch), whereas hyperalgesia is an increased sensitivity to a painful stimulus. Primary hyperalgesia caused by sensitization of C-fibers occurs immediately at the site of injury. On the other hand, secondary hyperalgesia develops in the uninjured surrounding area via changes in nociceptor activity on this region with/or sensitization of dorsal horn neurons. Neuropathic pain is generated by electrical hyperactivity of neurons along the sensory pathways consisting of at least three neurons. Changes in the expression of neuronal ion channels and its receptors, synaptic connectivity, and anatomy can contribute to formation of neuropathic pain (Basic-Kes et al. 2009). Due to the difficulty to identify specific neuropathic pain mechanisms, clinical investigations on pain mechanisms need specialized equipment. Because a simple focal peripheral nerve injury can trigger peripheral and central nervous pain system mechanisms. During inflammation or reparatory mechanisms of neural tissues responding to injury, the excitation threshold of nociceptors drops and lead to a state of hypersensitivity to stimulus, responsiveness to non-noxious stimulus and expanded receptive field, termed peripheral sensitization (Dworkin et al. 2003). At the same time, central neurons can undergo dramatic functional changes due to plasticity and including a state of hyperexcitability termed central sensitization (Woolf 2007; Loeser and Treede 2008). Normally these sensitization phenomena extinguish themselves as the tissue heals and inflammation subsides. Persistent injury or nervous system disease can modify the primary afferent function of the nervous system. Damage or permanent loss of primary afferent fibers, deafferentation, differentiates peripheral neuropathic pain from other types of pain (Dworkin et al. 2003). Positive sensory symptoms such as spontaneous pain, allodynia, and hyperalgesia are characteristics of patients with neuropathic pain. Their possible pathologic mechanisms can be ectopic generation of impulses, altered expression of neurotransmitters and their receptors, and changes in ion channels function. Direct injury of central structures may permanently alter sensory processing, and in some patients, it causes central neuropathic pain and dysesthesias (Dworkin et al. 2003). The mechanisms of central neuropathic pain are still unclear. In contrast, the mechanisms of nociceptive pain are more understandable and result from the direct activation of nociceptors in the skin or soft tissue in response to tissue injury and usually arise from accompanying inflammation. It is typically sensitive to anti-inflammatory agents, whereas neuropathic pain is poorly or not responsive to these medications. Also, both neuropathic and nociceptive pain can overlap and be caused by a complex mixture of both neuropathic and nociceptive factors. In this condition dysfunction of initial injury or nervous system may trigger the inflammatory reaction and subsequent neurogenic inflammation leading to mixed pain.

The primary aim of this study was to investigate plasma levels of PTX3 and TBP2 in diabetic neuropathy. We found that plasma PTX3 levels of the type 2 diabetic patients were higher than control group. Also the plasma PTX3 levels of type 2 diabetic patients with nociceptive pain were higher than type 2 diabetic patients with neuropathic pain and control group.

PTX3 is expressed in a variety of cells at inflammatory sites and is also stored in neutrophil-specific granules primarily in mononuclear phagocytes, fibroblasts and endothelial cells and may be an acute phase biomarker (Liu et al.

2014). Yang et al. (2014) found that PTX3 is positively associated with development and progression of diabetic retinopathy. The elevated plasma PTX3 levels may reflect the microvascular injury, and may contribute to systemic inflammatory diseases in type 2 diabetic patients.

According to our prediction, elevated plasma levels of PTX3 can trigger nociceptive pain. Nociceptive pain is caused by the stimulation of peripheral A-delta and C-polymodal pain receptors by mechanical, thermal and/or chemical stimuli. The chemical products of inflammatory cascade and immunological mediators can stimulate C-fibers (Giordano 2005). In the diabetic patients, tissue damage can cause the increase of plasma PTX3 levels, and the elevated PTX3 may enhance free H^+ concentrations, thereby inducing depolarization of C-fibers. Activation of C-fibers triggers release of substance-P known as a neurotransmitter of pain (Giordano 2005). Thus, PTX3 may have a role in transmission of nociceptive pain. PTX3 can be clinically helpful for the differentiation of the patients with neuropathic pain and nociceptive pain in type 2 diabetic patients.

Our results demonstrate that PTX3 may contribute to the onset of nociceptive pain in diabetic patients. These results should shed light on underlying mechanisms of the pathways leading to discrimination between neuropathic pain and nociceptive pain. Accordingly, PTX3 should be investigated in more detail with experimental studies *in vitro*.

Conflict of Interest

The authors declare no conflict of interest.

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