

**Bioinformatics analysis of diabetic neuropathy using functional protein sequences
reveals a dominant role for nerve growth factor**

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Abstract

Background: Diabetic neuropathy is an insidious complication of diabetes characterized by numbness and sometimes pain and weakness in the hands, arms, feet, and legs. It is most common among those who have had diabetes mellitus for at least 25 years. Diabetic neuropathy usually results from microvascular injury involving small blood vessels that supply nerves (vasa nervorum). Several studies suggested that lack of neurotrophic support is an important risk factor contributing to the etiology of diabetic neuropathy. In the present study, we evaluated the role of several proteins that are likely to be involved in diabetic neuropathy by employing multiple sequence alignment using ClustalW tool and constructed a phylogenetic tree using functional protein sequences extracted from NCBI. The phylogeny tree was constructed using Neighbor Joining Algorithm using bioinformatics principles and applications.

Results: Our results suggest a close association between nerve growth factor (NGF) protein and diabetic neuropathy. However, a role for aldose reductase, Na⁺-K⁺-ATPase, C-peptide, poly (ADP-ribose) polymerase (PARP), and erythropoietin is also evident from the present study, though their role appears to be relatively limited compared to NGF.

Conclusions: The association of NGF, aldose reductase, Na⁺-K⁺-ATPase, C-peptide, poly (ADP-ribose) polymerase (PARP), and erythropoietin in diabetic neuropathy suggests that a close interaction between these proteins may exist that may underlie the pathogenesis of diabetic neuropathy. The results of the present bioinformatics study indicate a predominant involvement of NGF in comparison to other proteins in the pathogenesis of diabetic neuropathy.

Background

Diabetic neuropathies are neuropathic disorders that are associated with diabetes mellitus. Diabetic neuropathy results from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum). Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. Neurologic problems in diabetes may occur in every organ system including the digestive tract, heart, and genitalia. People with diabetes can develop neuropathy at any time, but the longer a person has diabetes, the greater is the risk. It is known that about half of diabetics have some form of diabetic neuropathy, but not all with neuropathy have symptoms. The highest rates of neuropathy are among people who have had the disease for at least 25 years. Diabetic neuropathy is more common in people who have persistent hyperglycemia or poorly controlled diabetes, dyslipidemia and hypertension, and in people over the age of 40 with diabetes.

Diabetic neuropathies are classified as peripheral, autonomic, proximal, and focal. Peripheral neuropathy causes pain or loss of feeling in the toes, feet, legs, hands, and arms. Autonomic neuropathy causes changes in digestion, bowel and bladder function, sexual response, and perspiration and can also affect the nerves that serve the heart and control blood pressure. Proximal neuropathy causes pain in the thighs, hips, or buttocks and leads to weakness in the legs. Focal neuropathy results in the sudden weakness of one nerve, or a group of nerves, causing muscle weakness or pain. Diabetic neuropathy can affect any nerve in the body.

Diabetic neuropathy is mainly characterized by decline in nerve conduction velocity. Prolonged hyperglycemia produces neuropathological changes particularly in peripheral sensory nerves. Experimental studies revealed that lack of neurotrophic support is an important risk factor in the etiology of diabetic neuropathy. However, a role for ciliary neurotrophic factor (CNTF), aldose reductase, Na⁺-K⁺-ATPase, C-peptide, poly (ADP-ribose) polymerase PARP), nitrosative stress, that is, enhanced peroxynitrite formation, uncoupling proteins, and erythropoietin in diabetic neuropathy has also been reported in several experimental and clinical studies (1-17). In the present study, we evaluated the role of several proteins that are likely to be involved in diabetic neuropathy by employing multiple sequence alignment using ClustalW tool and constructed a phylogenetic tree using functional protein sequences extracted from NCBI. The phylogeny tree was constructed using Neighbor Joining Algorithm using bioinformatics principles and applications. The results of the present study reveal a close association between NGF protein and diabetic neuropathy.

Results

The functional protein sequences of 11 proteins that are believed to be involved in the pathogenesis of diabetic neuropathy collected from NCBI (National Center for Biotechnology Information <http://www.ncbi.nih.nlm.gov>) in FASTA forms (these sequences are given to clustalw <http://www.ebi.ac.uk/clustalw>) were analyzed for the multiple sequence alignment (it calculates that the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen) and the resultant score the phylogeny tree constructed based on these results are given in Table 1

and Figure 1 respectively. The phylogeny shows the distance between the protein sequences. The protein sequences with minimum distance are nerves growth factor-(3 polypeptide (NGFB), which suggests that this protein plays a significant role in the pathogenesis of diabetic neuropathy.

Discussion

Nerve growth factor (NGF) produced by cells of the skin, blood vessels and the bladder is taken up by the sympathetic and sensory fibres via a high affinity receptor (trk A) (18) Neurotrophins maintain the integrity of neurons owing to their ability to resist apoptosis and enhances regeneration of neurons. In experimental diabetic rats, reduction in NGF synthesis, secretion and reduced retrograde NGF transport in axons causing perturbations in the nerve conduction velocity have been described (1-3). Exogenous exposure to high levels of NGF increases the cellular content and release of substance P (19). On the other hand, neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and galanin (GAL) released from the cutaneous sensory nerve endings not only have neurotransmitter and immunoregulatory actions roles but also increase NGF mRNA expression and NGF secretion from keratinocytes in culture (20). In addition, SP, CGRP, VIP, and galanin increased the expressions of interleukin-1 α (IL-1 α), EL-8 and tumor necrosis factor- α (TNF- α) mRNA, suggesting that neuropeptides released from cutaneous nerves after an injurious stimulus upregulate cytokine and NGF expressions and secretion from the keratinocytes that may contribute to neural influence on skin health and disease. Hence, in instances where there is a

decrease in NGF synthesis causes deficiency of neuropeptides that, in turn, could contribute to the sensory nerve dysfunction (19). This is supported by the observation that treatment with NGF restored CGRP and SP in sensory neurons in the diabetic animals in a dose-dependent manner, suggesting that NGF can be effective in the prevention and treatment of diabetic sensory neuropathy (21). In addition to NGF, neurotrophic factors such as neurotrophin-3 and CNTF could also play a role in diabetic neuropathy since improved nerve conduction velocity has been documented in CNTF-treated experimental diabetic rats (4, 5).

Diabetic rats that showed a statistically significant reduction in H-wave-related sensory nerve conduction velocity (HSNCV) when treated with epalrestat, an aldose reductase inhibitor (ARI), resulted in a significantly greater NGF content and faster HSNCV than those in untreated diabetic rats, suggesting that aldose reductase inhibitors are useful in the treatment of diabetic neuropathy through NGF-induction (22). These results imply that a close interaction exists between polyol pathway and NGF secretion. It was noted that polyol pathway activity enhanced oxidative stress resulting in stimulation of NGF gene expression but not the expression of the analogous neurotrophin, neurotrophin-3 (NT-3), suggesting that oxidative stress and/or inflammation can drive up NGF expression in diabetes as a compensatory mechanism (23). In this context, it is interesting to note that aldose reductase enzyme is localized in Schwann cells. Increased production of NGF seen in Schwann cells treated with neurotrophin-3 (NT-3) was significantly suppressed when cells were cultured in the presence of high glucose. These Schwann cells also showed decreased levels of glutathione (GSH) and cAMP-response element binding protein (CREB) and elevated levels of activated nuclear factor-kappaB (NF-

kappaB), changes that were abolished when an AR inhibitor fidarestat was added. These results indicate that enhanced polyol pathway activity under high-glucose conditions suppressed NT-3-induced NGF production in Schwann cells, and thus, enhanced oxidative stress linked to the polyol pathway activity (24) may ultimately produce diabetic neuropathy.

Prolonged hyperglycemic state is known to suppress $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity (25), whereas the variant of the ATP1 A1 gene is considered as a predisposing factor for diabetic neuropathy (15). Previous studies revealed that increase in polyol accumulation is accompanied by decrease in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity and nerve conduction velocity (26). In contrast, NGF was found to enhance the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in pheochromocytoma cell nerve cells and chick embryo dorsal root ganglion cells in vitro (27, 28). In addition, there seems to be a close interaction between pro-inflammatory cytokines and NGF. For instance, both IL-6 and TNF- α enhance the expression and secretion of NGF (29, 30) whereas in trinitrobenzene sulfonic acid (TNBS)-induced model of colitis selective decrease among cholinergic excitatory neurons and calbindin-expressing neurons, and a trend to increase among inhibitory nitrenergic neurons suggesting that inflammation can give rise to a permanent imbalance between excitatory and inhibitory neural pathways with selective loss of NGF-sensitive neurons from inflammation-induced cell loss (31). Furthermore, EL-4, one of the Th2 cytokines, upregulated whereas IFN- γ , one of the Th1 cytokines, downregulated the expressions of NGF both in a time-dependent manner and in a dose-dependent manner, indicating that Th1/Th2 cytokine immune balance is influenced by NGF (32). These results imply a cytokine/neurotrophin axis in the pathogenesis of inflammation.

Thus, NGF seems to have the ability to modulate the concentrations of neuropeptides: substance P (SP), CGRP, VIP and galanin; participate in inflammation; regulate Na^+ - K^+ -ATPase activity, respond to plasma glucose and polyol concentrations, and has neuroprotective properties (see Figure 2) that suggests that therapeutic interventions aimed at enhancing its concentrations could be a promising approach in the prevention and treatment of diabetic neuropathy. This is supported by the results of the present bioinformatics study.

Material and methods

We collected 11 proteins that are believed to be involved in the pathogenesis of diabetic neuropathy. The functional protein sequence in FASTA forms for these proteins are collected from NCBI (National Center for Biotechnology Information <http://www.ncbi.nih.nlm.gov>). These sequences are given to clustalw <http://www.ebi.ac.uk/clustalw> for the multiple sequence alignment (it calculates that the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen). Based on these results, the score table and phylogeny tree are derived. The phylogeny shows the distance between the protein sequences. The protein sequences with minimum distance are nerves growth factor-(3 poly pep tide (NGFB).

Competing Interests

The author(s) declared that they have no competing interests.

Authors' Contributions

All the authors participated in the design of the study, interpretation of the results and preparation of the manuscript.

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S. no	Gene Name	Ac. No	Length	Tissue Type	Reference
1	AKR1B1	CAG29347	316aa	No Tissue Type	34 2
	ATP1A1	CAI15273	713aa	No Tissue Type	35
3	CNTF	AAH74964	200aa	Pooled tissue, PCR	4,36
	rescued clones 4	EPO	AAF23134	193aa	No Tissue Type
					12
5	NEF3	NP_005373	916aa	No Tissue Type	37 6
	NGFB	CAG46653	241aa	No Tissue Type	38 7 NTF3
	CAG46704	257aa	No Tissue Type	39 8	PARP1
	NP_001609	1014aa	No Tissue Type	8 9	TNFSF11
	CAM23721	244aa	No Tissue Type	40 10	UCP2
	AAC51336	309aa	skeletal muscle	17 11	C-Peptide
	1TOC_A	31aa	No Tissue Type	41	

Table.I: Table showing the genes/proteins that have been studied in the present study, which are believed to be involved in diabetic neuropathy.

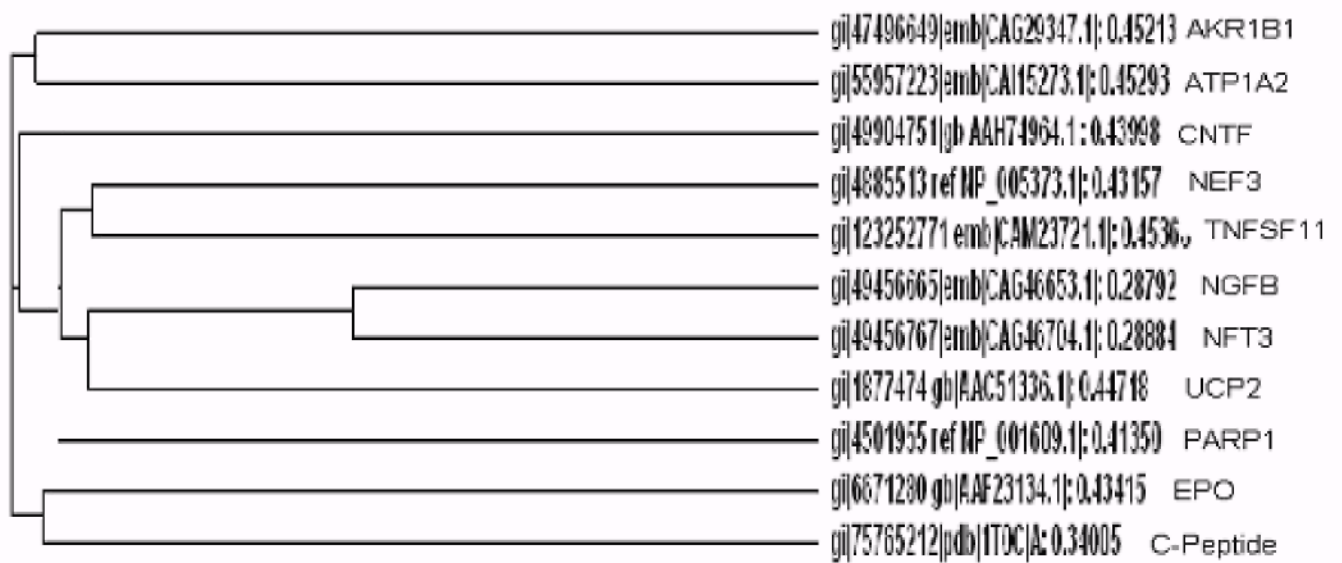


Figure 1. The phylogenetic tree constructed based on alignment scores of all the protein sequences involved in diabetic neuropathy. A high degree of homology was noted for NGFB.

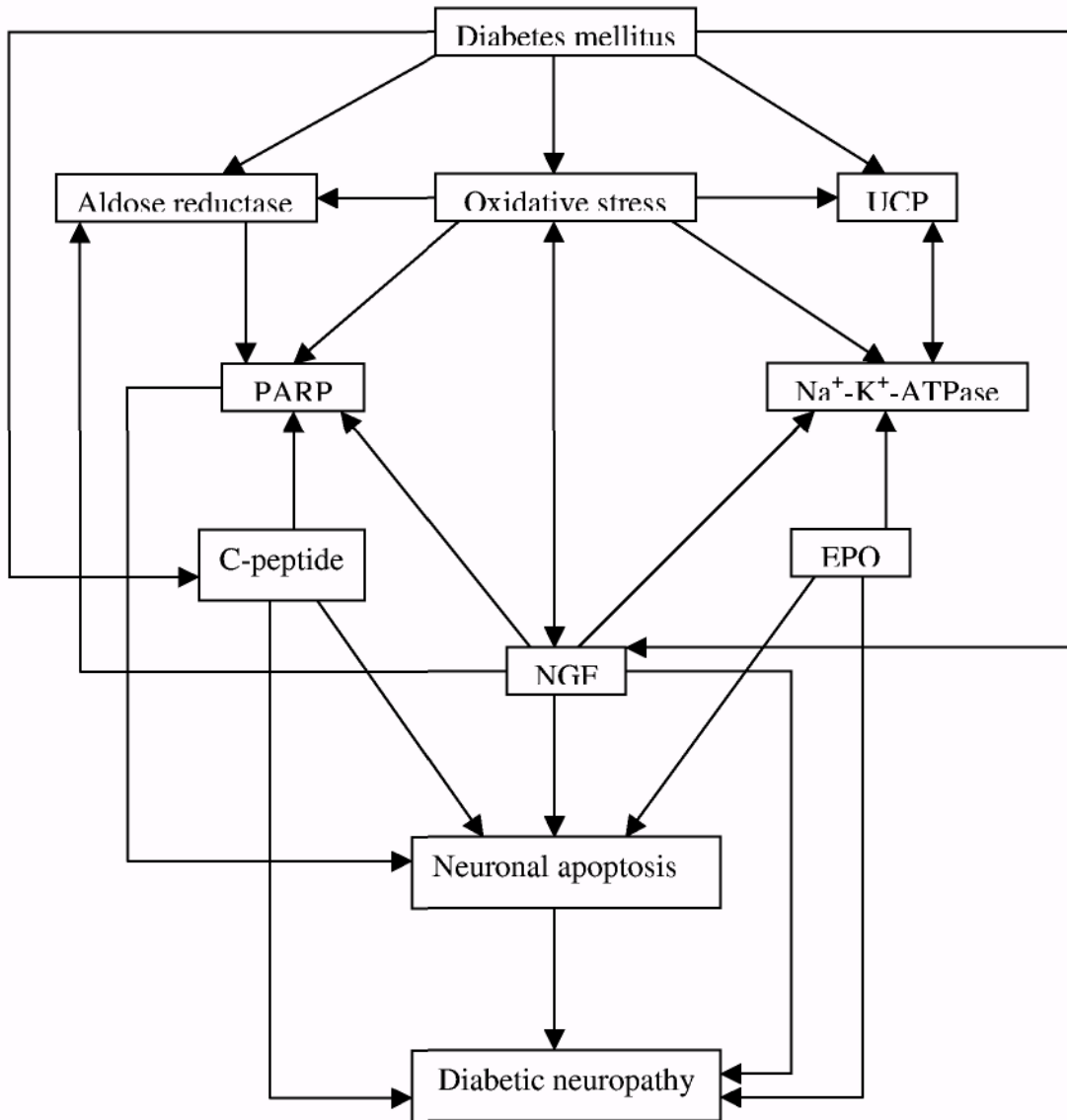


Figure 2: Scheme showing possible role of various factors involved in the pathogenesis of diabetic neuropathy and their interactions.

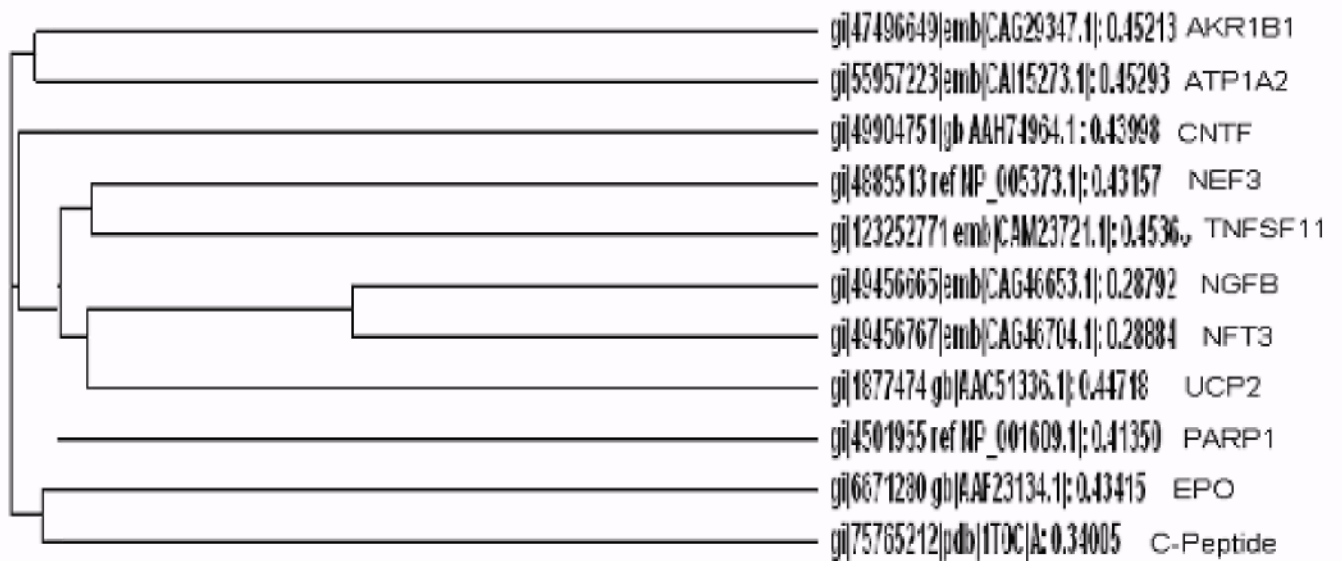


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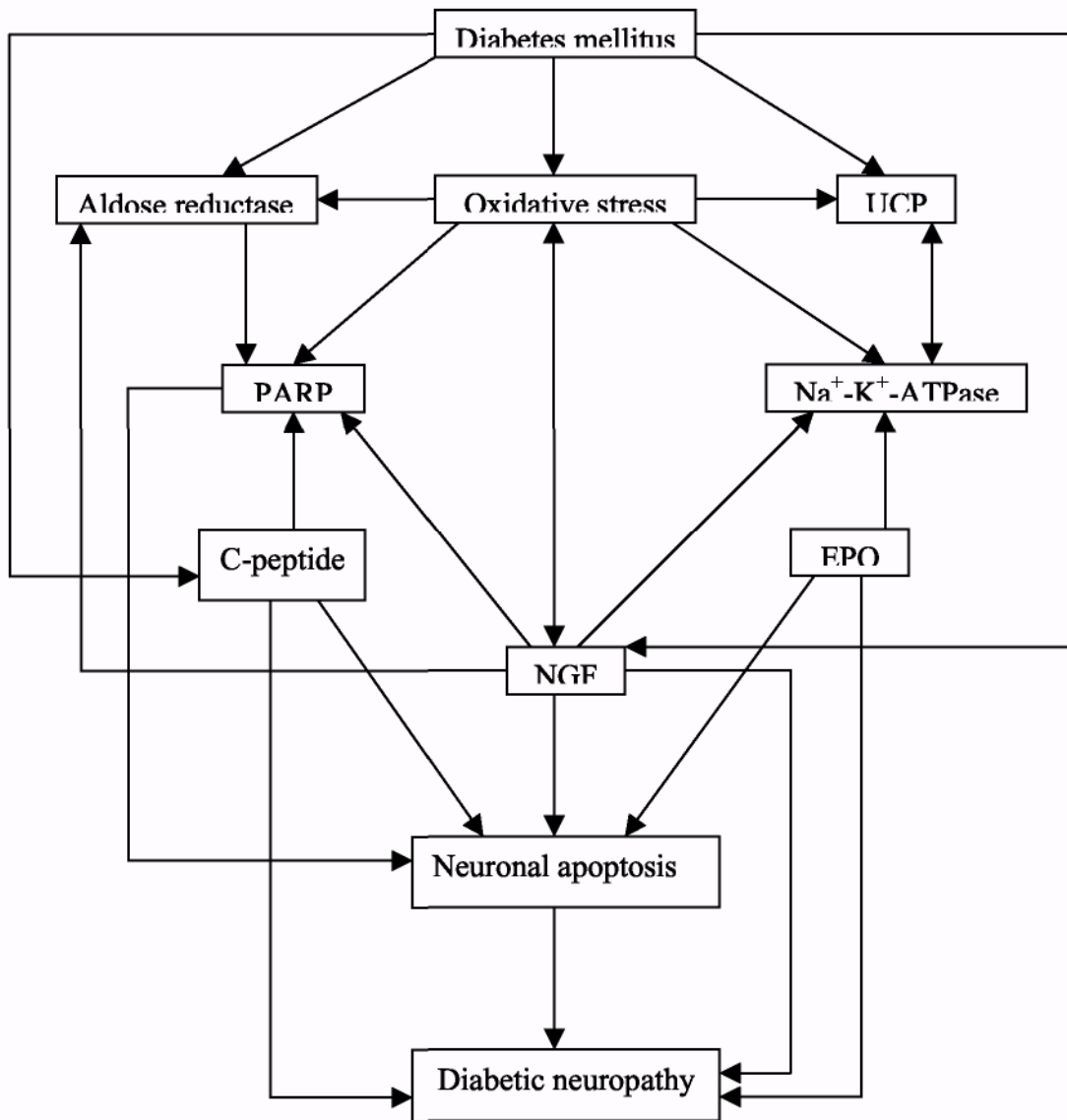


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