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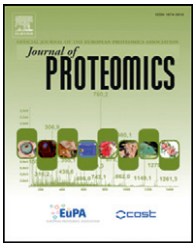

Proteomic profiling of the substantia nigra demonstrates CNDP2 overexpression in Parkinson's disease☆

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ABSTRACT

Despite decades of intensive investigations, the precise sequence of molecular events and the specific proteins mediating the degenerative process underlying Parkinson's disease (PD) remain unraveled. Proteomic strategies may provide unbiased tools to identify novel candidates and explore original mechanisms involved in PD. Substantia nigra pars compacta (SN) tissue, whose degeneration is the hallmark of PD, was dissected from neuropathologically confirmed PD patients ($n=3$) and control subjects ($n=3$), before being submitted to a comparative 2-DE analysis. The present study revealed a subset of neuronal and/or glial proteins that appears to be deregulated in PD and likely to contribute to neurodegeneration. Observed alterations not only consolidate well accepted concepts surrounding PD pathogenesis such as oxidative stress and mitochondrial dysfunction but also point out to novel pathways. Among the latter, cytosolic non specific dipeptidase 2 (CNDP2), a relatively unknown protein not yet reported to be associated with PD pathogenesis, was shown to be increased in the SN of PD patients, as confirmed by Western blot. Immunohistochemical analyses demonstrated the presence of CNDP2 within the cytoplasm of SN dopaminergic neurons. Altogether, our findings support a key role of CNDP2 in PD neurodegeneration, by mechanisms that could involve oxidative stress, protein aggregation or inflammation. This article is part of a Special Issue entitled: Translational Proteomics.

Abbreviations: CNDP2, cytosolic non specific dipeptidase 2; CSF, cerebrospinal fluid; DA, dopamine; LB, Lewy body; PD, Parkinson's disease; SN, substantia nigra pars compacta; UKPDSBB, United Kingdom Parkinson's disease society brain bank; WB, Western blot.

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1. Introduction

Parkinson's disease (PD) is a common and devastating neurodegenerative disorder caused by the progressive loss of pigmented dopaminergic (DA) neurons in the substantia nigra pars compacta (SN) [1,2]. Lewy bodies (LB) – cytoplasmic inclusions of aggregated proteins – and Lewy neurites are observed in the surviving nigral neurons. Setting aside a minority of PD cases who carry identified genetic mutations or who have been exposed to neurotoxins such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydroptyridine (MPTP) [3], the primary cause of PD neurodegeneration remains to be established. Sporadic PD is likely to result from the subtle interplay between ageing, a predisposing genetic background and environmental toxic factors, which converge to initiate a cascade of still undetermined molecular events ultimately leading to cell death. Motor symptoms of PD (i.e. bradykinesia, rigidity or tremor) develop when about 60–70% of nigral neurons are lost [4]. Therefore, in the absence of early biomarkers, PD diagnosis is still based on clinical manifestations that develop at an advanced neuropathological stage. So far, no treatment is effective to cure PD, and DA replacement therapies only provide a symptomatic relief at the expense of severe side effects. The limited understanding of PD pathogenesis and the lack of reliable biomarkers constitute major hurdles for the development of strategies able to slow or halt disease progression.

In the last decades, genetic studies have provided some aid in understanding the mechanisms underlying the condition. For example, genes harboring mutations causally related to rare monogenic forms of PD have suggested that deregulations in alpha-synuclein (α-SYN, *SNCA*) metabolism, mitochondrial functions (*PINK1*, *DJ-1*, *LRRK2*), protein degradation (*Parkin*, *UCHL-1*) or antioxidant (DJ-1) systems (reviewed in [5]) may be central in PD pathogenesis. The involvement of those proteins or pathways in sporadic PD, which represents about 90% of all PD cases, was further substantiated by various observations. Recently, genome wide association studies have provided evidence that common variants in several genes – including some known from inherited PD subtypes (i.e. SNCA or LRRK2) and others (i.e. MAPT) – were conferring an increased susceptibility to develop sporadic PD [5]. Examination of sporadic PD patients' SN showed a reduced activity of the ubiquitin proteasome system (UPS) and mitochondrial complex I as well as higher concentrations of oxidized proteins [6,7]. Importantly, LBs in sporadic PD brains are strikingly positive for α-SYN and ubiquitin [8,9]. Whether LB themselves cause neurodegeneration is still unclear, but abnormal accumulation of unwanted proteins and failure of the UPS to degrade them, successively leading to protein oligomerization and aggregation, appears central to cell demise. Furthermore, growing evidence has suggested that protein misfolding, mitochondrial dysfunction and altered autophagy of mitochondria, oxidative stress, energy production imbalance, excitotoxicity, inflammation, defects in neurotrophic factors, or apoptosis are all contributing factors. However, neither unifying nor completely satisfying hypotheses on PD pathogenesis have been established yet.

Mainly driven by genetic discoveries, candidate-based research focusing on selected pathophysiological pathways has failed to decipher sporadic PD mechanisms. In this setting, hypothesis-free strategies using novel high-throughput "omics" technologies may provide unbiased exploratory tools to revisit the pivotal issues described above and identify novel molecular pathways without relying upon pre-existing or *a priori* pathogenic hypotheses. Because proteins are the major determinants of the diversity of phenotypes arising from a common set of genes and because sporadic PD can be essentially viewed as a disorder of protein handling, proteomics-based analysis might be one of the most appropriate strategy to approach PD pathogenesis.

A limited number of proteomic studies investigating human *post-mortem* SN has been published so far in the field of PD research [10–13]. In the absence of fully satisfying PD animal models, human autopsy tissues represent a unique opportunity to highlight PD specific abnormalities. This approach has been recently reappraised with the demonstration that only a minority of proteins undergoes massive degradation after a prolonged *post-mortem* delay of 72 h at room temperature [14]. The rationale for a whole tissue approach is provided by the fact that PD is an insidious disorder involving not only neurons but also their environment, within and beyond the SN, where different subpopulations of glial cells (microglia, astrocytes, oligodendrocytes) may contribute actively to DA neuronal death [15].

In this study, SN was selectively dissected from PD patients' and controls' autopsy tissues and extracted proteins were submitted to two-dimensional gel electrophoresis (2-DE). We delineated a subset of deregulated neuronal and glial proteins in PD which may participate to the pathological processes related to PD. Observed alterations consolidate some popular theories such as oxidative stress and mitochondrial dysfunction but also point out to yet unidentified pathways. Novel potentially pathogenesis-relevant candidates were identified, such as the cytosolic non specific dipeptidase 2 (CNDP2) which appears to be overexpressed in PD nigral neurons.

2. Materials and methods

2.1. Human samples

2.1.1. Brain tissues

Mesencephalon specimen from six PD patients and four control subjects were collected at autopsy in the Division of Clinical Pathology of the Geneva University Hospitals under an ethically approved protocol and either frozen at −80 °C or fixed in 15% formaldehyde for 4 weeks at 4 °C before being paraffin-embedded (Table 1). An informed consent form was signed by close relatives to proceed with the protocol research. PD final diagnosis was confirmed *post-mortem* by neuropathological examination assessing the presence of two pathological hallmarks of the condition, i.e. severe neuronal loss in the SN and presence of α-SYN immunoreactive inclusions (LB). Controls were cases with no previous history of any neurological or psychiatric disorders and no nigral abnormalities. For all experiments, patients from both groups were matched as closely as possible with respect to age, gender and post-mortem interval (<34 h).