

# QUANTITATIVE PROTEOMICS OF MITOCHONDRIAL PROTEINS IN A MOUSE MODEL FOR SPASTIC PARAPLEGIA

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## **Abstract**

Quantitative studies of the mitochondrial proteome in a mouse model for hereditary spastic paraplegia SPG13 due to mutation in the HSPD1 gene, which encodes the mitochondrial chaperone Hsp60, is applied to get insights into the molecular pathogenesis. By assessing the mitochondrial proteome of mutant animals compared to controls in different tissues the molecular disease mechanisms and the rationale for tissue specific manifestation can be pinpointed.

**Keywords: quantitative proteomics, mitochondria, neurodegeneration**

## **1. Introduction**

At least two different mutations in the HSPD1 gene, which encodes the Hsp60 protein, cause an autosomal dominant form of hereditary spastic paraplegia [1-3]. Spastic paraplegia is due to selective loss of function of neurons in the central nervous system possessing the longest axons in the body and manifests as a ‘dying-back’ from the axon ends [4-7]. Based on knowledge on more than 30 different gene loci associated with the disease, it has been hypothesized that the molecular mechanisms involved in the neurodegenerative process are either related to maintenance of mitochondrial function or to deficits in intracellular transport processes [7]. Homozygosity for a mutation in Hsp60 has recently been shown to cause a fatal hypomyelinating leukodystrophy [8].

Such patients present with a variety of neurological features including spastic paraplegia. Based on our studies on the effects of the disease-associated Hsp60 mutations [8,9], we suggest that the residual levels of Hsp60 activity inversely correlate to severity of the neurodegeneration.

## **2. Theory**

Hsp60 belongs to a group of molecular chaperones that associate with co-chaperones to form ring structures with an inner cavity, thus providing a secluded environment for folding of substrate proteins. Hsp60 transiently binds certain newly synthesized and stress-denatured proteins and mediates their folding or refolding into the functional 3-dimensional conformation. The genes encoding the mitochondrial Hsp60 chaperone or its homologs are essential in bacteria, yeast, fruit fly [10-12] and, as shown recently by us, in mammals [13]. This is due to the fact that a subset of proteins are unable to fold efficiently by assistance of other molecular chaperones. Our hypothesis is that deficiency of Hsp60 leads to inefficient folding or misfolding of a number of mitochondrial proteins resulting in their degradation by the protein quality control system. Deficiency in the activities and functions of these proteins triggers neurodegeneration in the affected neurons. The mitochondrial proteome with an estimated 1000-1500 different proteins can be addressed by modern mass spectrometry. By quantitative mass spectrometry of the mitochondrial proteome we wish to identify proteins affected by decreased Hsp60 activity as they display reduced levels as well as proteins whose levels is altered due to compensatory regulatory mechanisms. As mitochondrial dysfunction is a common finding in neurodegenerative processes, our findings may identify proteins and pathways, which play an important role in other neurodegenerative diseases.

## **3. Experimental**

Mice heterozygous for an inactivating genetrap insertion in the murine *hspd1* gene and corresponding wild type controls at different ages were sacrificed and brain and liver tissues were isolated. Tissue samples were lysed and mitochondria enriched by differential centrifugation. The mitochondrial protein enrichments were digested with trypsin and tagged with different iTRAQ labels. Labelled peptides from mutant and control mice were mixed, fractionated by isoelectric focussing. Peptide fractions were separated by liquid chromatography (Easy nLC from Proxeon) coupled coupled to a mass spectrometer (LTQ-Orbitrap, Thermo Fisher Scientific) through a nano-electrospray source. Processed raw data were searched with Mascot (Matrix Science).

#### 4. Results and discussion

Using the described approach, we typically detected and quantitated several hundred mitochondrial proteins. So far liver, brain and brain cortex, the tissue in which the cell bodies of the affected neurons are localised, have been analysed. In most tissues Hsp60 was reduced to approximately 50-60% as expected. Our preliminary results indicate that proteins involved in oxidative stress response, respiratory chain activity and mitochondrial fission and fusion are affected by Hsp60 deficiency.

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