# 2. BIOMARCADORES Y PATOLOGÍAS HUMANAS

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## Protein targets of oxidative stress induced by Huntington disease in human brain

### Evaluation of an HD mice model: Tet/HD94

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Huntington disease (HD) is an inherited neurodegenerative disorder characterized by degeneration of neurons affecting the striatum and the cortex. The disease involves expansion of CAG trinucleotide repeats in the *huntingtin* gene codifying for glutamines in the htt protein [1].

The main goal of this work is to study protein oxidation in post-mortem tissue samples (striatum) from individuals affected of HD. Moreover, a conditional HD mouse model is used to evaluate whether similar proteins are affected in these mice (Tet/HD94) [2]. These mice express a polyQ sequence of 94 glutamines under the control of a doxycycline-regulatable promoter and resembles the human phenotype.

Protein oxidation by reactive oxygen species can generate carbonyl groups on the side-chain of several amino acids and these groups can be derivatized by 2,4-dinitrophenylhydrazine (DNPH) and detected by Western blot [3]. We performed a 2D-gel electrophoresis analysis followed by anti-DNP Western blot in order to identify oxidized proteins in human brain post-mortem samples obtained from striatum of HD patients compared to samples of age and sex-matched controls (Figure 1). A comparison between 8 control-HD pairs was made and spots that showed a ratio of oxidation level HD/control > 1.5 (once normalized for protein amount) were identified by PMF or MS/MS (Table 1). The most interesting result was the oxidation of pyridoxal 5-phosphate kinase, potentially leading to a reduction in PLP availability. PLP is a cofactor of enzymes involved in neurotransmitter metabolism (GABA production).

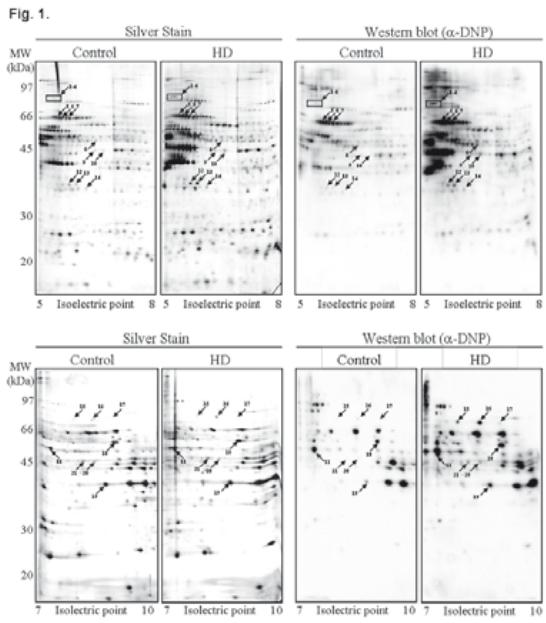


**Figure 1.** *Oxyproteome analysis of human samples. Comparative analysis revealed several spots with increased oxidation levels in HD. Identification was performed and proteins listed in Table 1.*

The results from Tet/HD94 mice show that protein oxidation is increased in HD94 expressing mice (gene on) in striatum, while no differences are observed in cortex and cerebellum (Fig 2). Also, there

**Table 1.** *Identification of oxidized proteins in HD.*

| SPOT | PROTEIN | GENE | ACCESSION NUMBER | MOLECULAR MASS (DA) | *OXIDATION |
|---|---|---|---|---|---|
| 1 | **Transitional endoplasmic reticulum ATPase (VCP)** | VCP | P55072 | 89322 | 112,76 |
| 2 | | | | | 9,02 |
| 3 | | | | | 423,73 |
| 4 | | | | | 50,50 |
| 5 | **Heat shock cognate 71 kDa protein** | HSC71 | P11142 | 70898 | 7,38 |
| 6 | | | | | 1,64 |
| 7 | | | | | 2,53 |
| 8 | **α-aminoadipic semialdehyde dehydrogenase** | ALDH7A1 | P49419 | 55332 | 3,40 |
| | **Cytosol aminopeptidase** | LAP3 | P28838 | 56131 | |
| | **T-complex protein 1 subunit beta** | CCT2 | P78371 | 57452 | |
| 9 | **α-enolase** | ENO1 | P06733 | 47169 | 5,41 |
| 10 | | | | | 1,79 |
| 11 | | | | | 4,88 |
| 12 | **N(G), N(G)-dimethylarginine dimethylaminohydrolase1** | DDHA1 | O94460 | 31122 | 1,90 |
| 13 | | | | | 1,82 |
| 14 | **Pyridoxal kinase** | PDXK | O00764 | 35080 | 2,42 |
| 15 | **Pyruvate kinase isoenzymes M1/ M2** | PKM2 | P14618 | 57937 | 47,01 |
| 16 | | | | | 15,26 |
| 17 | | | | | 4,86 |
| 18 | **ATP synthase subunit alpha** | ATP5A1 | P25705 | 59751 | 2,32 |
| 19 | **Glyceraldehyde-3-phosphate dehydrogenase** | GAPDH | P04406 | 36053 | 2,28 |
| 20 | **Cytochrome b-c1 complex subunit 2, mitochondrial** | UQCRC2 | P22695 | 48413 | 7,93 |
| 21 | **Citrate synthase, mitochondrial** | CS | O75390 | 51680 | 7,51 |
| | **Creatine kinase, ubiquitous mitochondrial** | CKMT1A | P12532 | 47007 | |
| | **Creatine kinase, sarcomeric mitochondrial** | CKMT2 | P17540 | 47474 | |
| | **Fructose-biphosphate aldolase A** | ALDOA | P04075 | 39395 | |

**The oxidation level is estimated as a ratio between HD and control oxidation signal, divided by the ratio of their protein amount.*

is an evident decrease in the pyridoxal 5-phosphate kinase levels in the HD94 expressing mice (gene on), that is recovered after 5 months of doxycycline treatment (gene off), reaching the levels of control mice. Moreover, this protein seems to be more oxidized in the "gene on" mice (Figure 3).

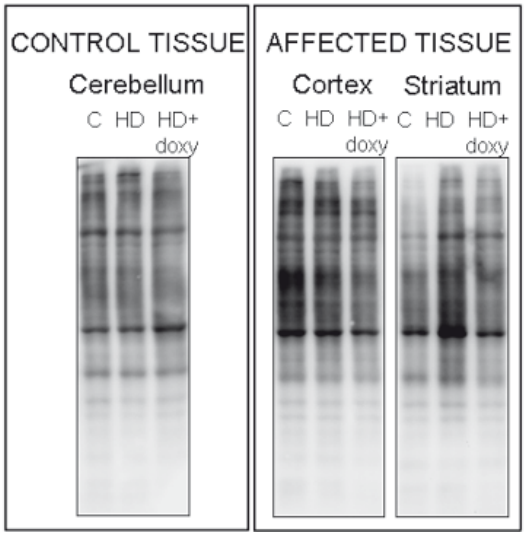


**Figure 2.** *Protein oxidation in Tet/HD94 mice. All mice were 24 months old. C: control mice; HD: "gene on" mice; HD+doxy: "gene on" for 19 months + "gene off" for 5 months (doxycycline treatment).*

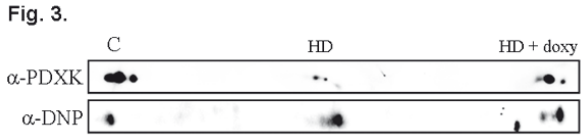


**Figure 3.** *Levels and oxidation of pyridoxal 5-phosphate kinase in Tet/HD94 mice. Amplified sections of western blot anti-PDXK and anti-DNP from 2D gel separation of tissue extracts from striatum of Tet/HD94 mice.*

As a conclusion, the human oxyproteome experiments show a clear energetic failure and PLP metabolism disruption. Moreover, these evidences are also observed in the mice model, leading to the hypothesis that possible interventions to increase PLP availability and energy production can be taken in account.

## References

[1] Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72: 971-983.

[2] Yamamoto Ai, Lucas JJ and Hen R. Reversal of Neuropathology and Motor Dysfunction in a Conditional Model of Huntington disease. Cell 2000; 101:57-66.

[3] Levine RL, Williams JA, Stadtman ER and Shacter, E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 1994; 233: 346-357.