Can cumulation of neurodegenerative disorders significantly promote mitochondrial dysfunction in cultured skin fibroblasts?

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Introduction

In elderly patients combination of different neurodegenerations could be seen quite often as "overlap syndrome". But combination of Alzheimer´s disease (AD) symptoms, combined with Huntington´s Disease (HD) and fully evolved Multiple system atrophy (MSA) diagnosed in our proband is unique.

Mitochondrial impairment plays important role in pathogenesis and progression of HD and also other neurodegenerative disorders.

Our Aim was to analyze mitochondrial impairment level in fibroblasts from the patient with unique combination of late onset HD and fully evolved MSA and AD.

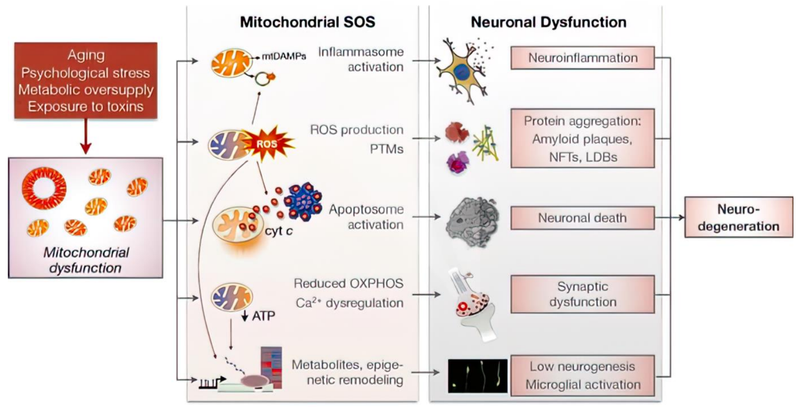

Figure 1: ***Stress signals: from mitochondrial dysfunction to neurodegenerative disease****. Molecular changes within the cytoplasm and nucleus that directly contribute to the established hallmarks of neurodegenerative disease, including protein aggregates and transcriptional dysregulation (*Picard M., McManus M.J. (2016)

Case report

67-year old woman (negative family history) developed slowly over two years gait disturbance, dysarthria, writing difficulties, increasing nervousness, anxiety and irritability. Clinical testing showed mild generalized choreatic syndrome, bilateral atrophy of the head of the caudate nucleus. Genetic testing confirmed HD (17/40 CAG triplets).

Two years later, the patient progressed significantly within a short time (hyperactive dysarthria, gait instability with ataxia and dizziness, repeated falls without loss of consciousness, urinary incontinence, neurogenic orthostatic hypotension, oedema of lower limbs, impairment of swallowing, weight loss, hypofonic and mostly incomprehensible speech) At the nursing home she became confused with important memory and orientation difficulties. She died from terminal bronchopneumonia at the age of 74 years. Autopsy showed changes in brain tissue corresponding to AD and MSA at the same time, which could have caused a sudden worsening of the disease

Huntington´s disease (HD) is autosomal dominant neurodegenerative disorder caused by pathological expansion of CAG triplet in gene for protein huntingtin. HD manifests usually between 30 and 50 years by chorea, neuropsychiatric symptoms, and progressive cognitive deterioration. Patients are fully dependent on care from their relatives in later stages of the disease. Intensive research of HD pathology in last decades showed mitochondrial dysfunction plays important role in disease progression.

Alzheimer's disease (AD) is the commonest neurodegenerative disease and the most frequent cause of dementia. It affects 30 million people worldwide is the most common cause of dementia in old age. It causes a decline in thinking, memory, and language, personality changes. Neuropathology is associated with extraneuronal toxic amyloid oligomers and intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau, region-specific diminished cerebral glucose metabolism, synaptic dysfunction, and mitochondrial dysfunction.

Multiple system atrophy (MSA) is a sporadic, progressive neurodegenerative disorder with onset in adulthood and diverse clinical manifestations, including parkinsonism, cerebellar syndrome, and autonomic failure. Neuropathological hallmark of MSA is characterized by glial cytoplasmic inclusions in oligodendrocytes, which contain fibrillary forms of α-synuclein. Changes in mitochondrial respiratory chain and impaired apoptotic signaling were observed in MSA model cell lines (Monzio et al., 2018). The mean life expectancy of MSA is 6–10 years following diagnosis.

Results

Postmortal morphological analysis of selected cerebral areas

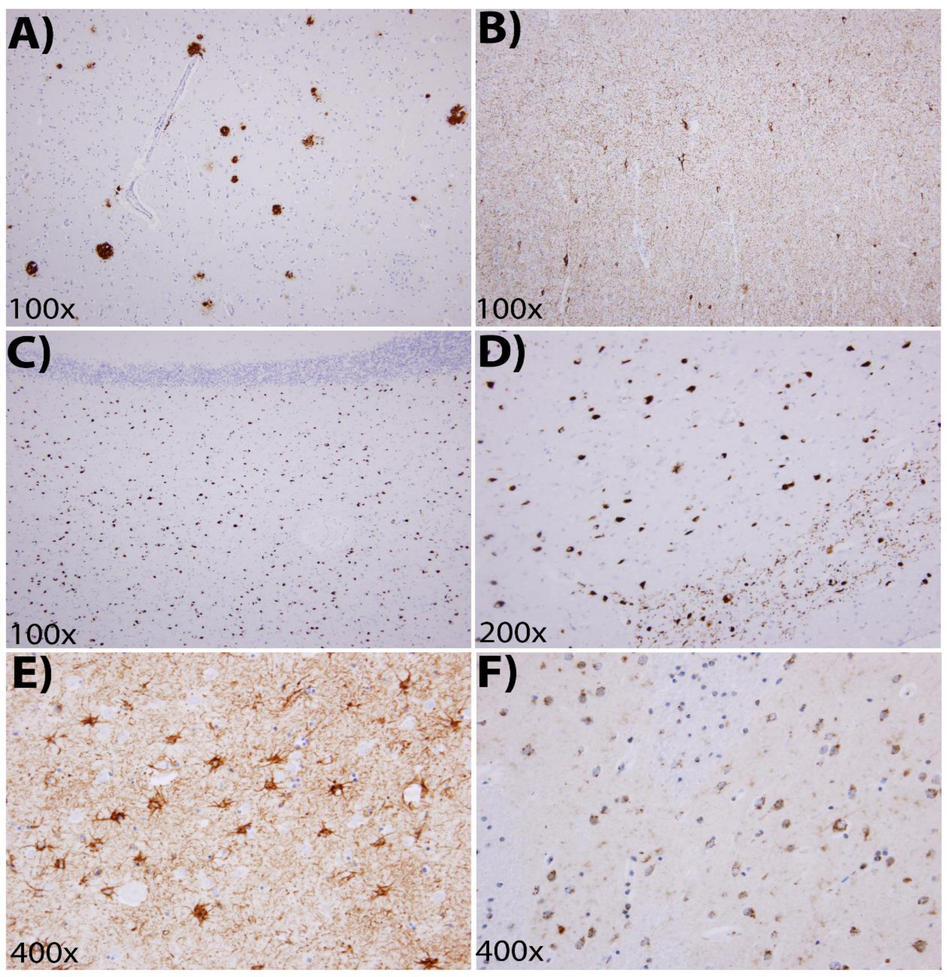

Figure 2: ***Neuropathological findings on the patient's autopsy: A) Varied morphology of amyloid plaques in the striatum****. The plaques stain for immunohistochemical reaction with monoclonal antibody against amyloid-ß-peptide. Original magnification 100X.* ***B) Numerous neurofibrillary tangles,*** *stained with monoclonal antibody against hyperphosphorylated tau protein (clone AT8) in the temporal cortex. Original magnification 100X.* ***Multiple Papp-Lantos glial cytoplasmic inclusions specific for MSA,*** *positive in immunohistochemical staining using monoclonal anti-body against alpha-synuclein (clone 5G4) in the cerebellum* ***(C)****, original magnification 100x and in the pons Varoli* ***(D)****, original magnification 200X.* ***E) Prominent astrogliosis in the neostriatal region*** *demonstrated by polyclonal anti-GFAP immunohistochemical staining. Original magnification 400X.* ***F) Abundant cytoplasmic granules in neurons of neostriatal region*** *demonstrated using immunohistochemical reaction with antibody against polyQ repeats (clone 3B5H10). Original magnification 400X.*

Analysis of mitochondrial structure and functions in fibroblasts from the patient and control

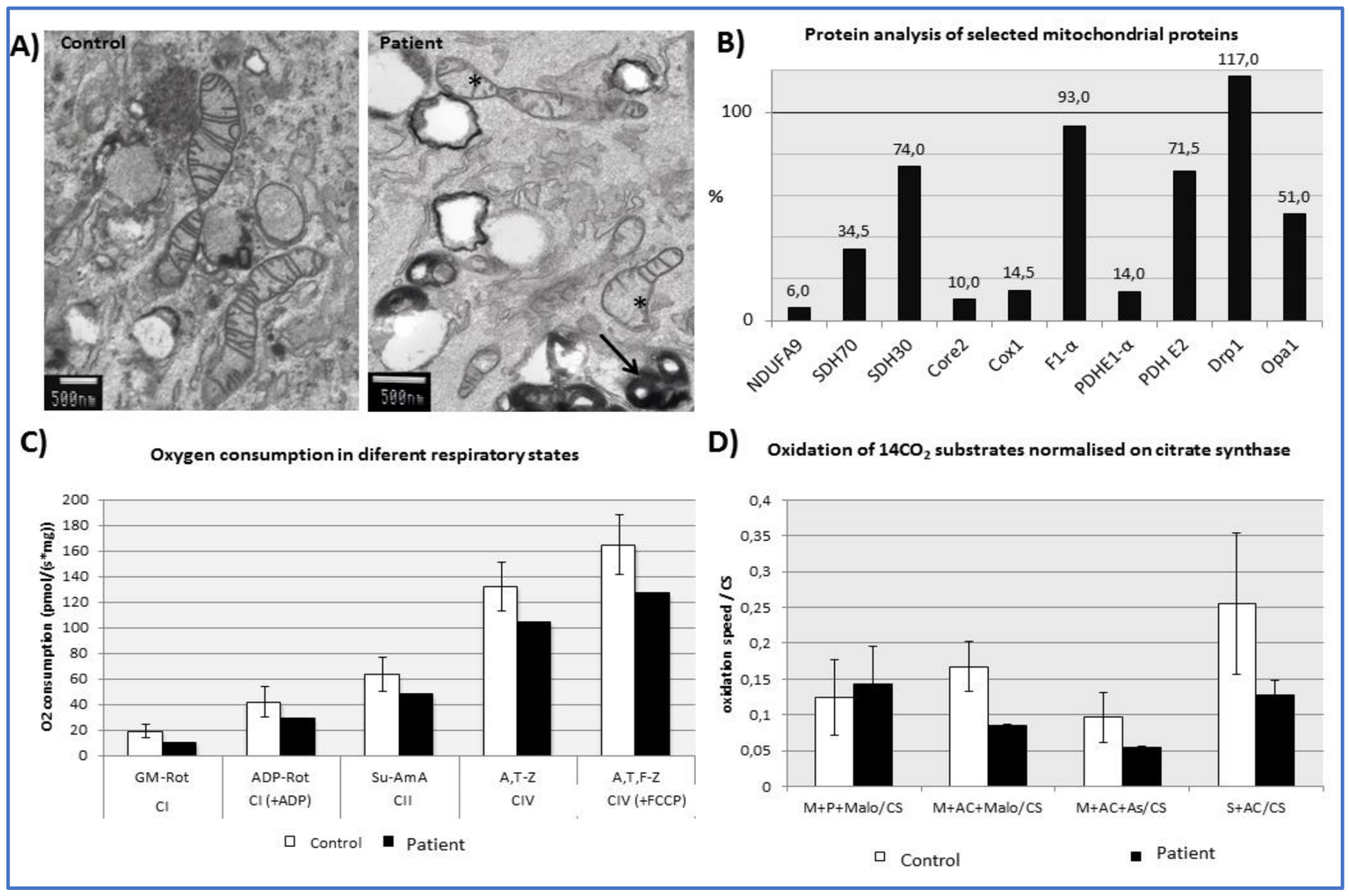

Figure. 3: ***Structural (A, B) and functional (C, D) abnormalities detected in fibroblasts from our patient in comparison to controls. A) Pathological changes of mitochondrial ultrastructure visualized by TEM*** *(Jeol JEM 1200). Original magnification: 15 000x,* ****decreased level of cristae, ↓ cellular/mitochondrial degradation bodies are showed. B) Evaluation of protein analysis of selected mitochondrial proteins of OXPHOS system, PDH complex and Drp1 and Opa1.*** *Cell lysates of patient´s and control fibroblasts were separated by SDS-PAGE, proteins were transfered to PVDF membrane and detected by monoclonal antibodies (Abcam). Results were quantified by Quantity one programe and normalised to tubulin. Control Value corresponds to 100% and it´s an average of 2 adult contol samples.* ***C) Decreased oxygen consumption parameters in various respiratory conditions measured using Oroboros Oxygraph****, folowed pyruvate protocol. GM-glutamate, malate, ADP-adenosintriphosphate, rot-rotenon, succ-succinate, ama-antimycin A, aT- ascorbate + TMPD, aTF- ascorbate + TMPD + FCCP, asctmpd-ascorbate + TMPD. Control value corresponds to average value of measurement of 3 adult controls.* ***Respiration in patient´s fibroblasts is decreased after adding of substrates for all respiratory chain complexes, which suggests generalised deficiency of OXPHOS metabolism. D) Significantly lower values of mitochondrial energetic metabolism capacity.*** *M+P+Malo - [U-^{14}C] malate + acetylcarnitine + malonate; M+AC+Malo- [U-^{14}C] malate +acetylcarnitine + arsenite, S+AC+Malo-[1,4-^{14}C]succinate + acetylcarnitine. All rates were normalized to CS-citrate synthase, N of controls=3.* ***Decreased oxidation in displayed reactions indicate deficit of Krebs cycle and decreased function of complex II (succinate dehydrogenase)*** *.*

Conclusions

Results confirmed structural and functional changes of mitochondria in fibroblasts from patient. Mitochondrial disruption was more propagated in our proband then in fibroblasts of 10 HD patients group analyzed in parallel (Vanisova et al. In preparation). We suppose, combination of three neurodegenerative diseases, could be the reason of the profound mitochondrial phenotype.