

Short Communication

Buccal Respiratory Chain Complexes I and IV Quantities in Huntington's Disease Patients

(Huntington's disease / mitochondria / buccal epithelium / OXPHOS / dipstick immunoassay)

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Abstract. Alterations in mitochondrial parameters are an important hallmark of Huntington's disease (HD). The ubiquitous expression of mutant huntingtin raises the prospect that mitochondrial disturbances can also be detected and monitored through buccal epithelial cells. In a group of 34 patients with Huntington's disease and a group of 22 age-related healthy volunteers, respiratory complex I and IV protein quantities in buccal epithelial cells were measured using the dipstick immunocapture assay. The protein quantity of respiratory complex I correlates with age ($r = 0.427$, $P = 0.026$, $FWE-P = 0.156$) in the patient group, but not in the group of healthy subjects. Our non-invasive approach allows us to obtain valuable information for the studies of mitochondrial biochemical parameters in patients with neurodegenerative diseases and could also be useful in epidemiological studies.

Introduction

Huntington's disease (HD) is an autosomal dominantly inherited, incurable and devastating neurodegenerative

disease with an incidence of 0.38 per 100,000 per year, and prevalence of 0.4–7.33 per 100,000 inhabitants (Rawlins et al., 2016). It is caused by a mutation leading to multiplication of a cytosine-adenine-guanine (CAG) trinucleotide (triplet) at chromosome 4 (4p16.3) exceeding 39 repeats (Cardoso et al., 2006). The product of the mutation is an aberrant protein called huntingtin (Htt) with an enlarged polyglutamine stretch (Hoogeveen et al., 1993).

HD manifests by neurological (voluntary movement impairment and involuntary movements), psychiatric (cognitive impairment and behavioural abnormalities) and somatic signs (cachexia and cardiomyopathy) (Mielcarek et al., 2014; Labuschagne et al., 2016; Cardoso, 2017; Profant et al., 2017).

Mitochondrial dysfunction is suggested as one of the mechanisms that plays an important role in the pathogenesis of neurodegenerative disorders. In HD, several mechanisms of disturbance of mitochondrial functions have been described, e.g., direct inhibition of *PGC1 α* gene expression (Cui et al., 2006), calcium level dysregulation (Panov et al., 2002), oxidative stress (Tabrizi et al., 1999), impaired mitochondrial fission and transport (Kim et al., 2010), and particularly disruption of oxidative phosphorylation (OXPHOS).

OXPHOS impairment in HD has been found in many tissues, e.g., caudate nucleus (Brennan et al., 1985; Gu et al., 1996), putamen (Browne et al., 1997), cerebral cortex (Tabrizi et al., 1999), and blood platelets (Klempíř et al., 2005; Silva et al., 2013). In model organisms, mutated huntingtin (mHtt) caused alterations in respiratory chain complexes (RC) II and III (Solans et al., 2006; Damiano et al., 2013).

Peripheral tissue readily available by non-invasive or minimally invasive sampling that would bear similar mitochondrial changes as within neuroectodermal brain structures would be useful to study or monitor changes in groups or individuals. Two studies investigated mesenchymal tissues; however, neither found any correlations with the parameters measured in the central nerv-

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Abbreviations: BMI – body mass index, CAG – cytosine-adenine-guanine, CS – citrate synthase, DIA – dipstick immunocapture assay, FWE – family-wise error, HD – Huntington's disease, Htt – huntingtin, mHtt – mutated huntingtin, Ndi1 – NADH dehydrogenase internal 1, OXPHOS – oxidative phosphorylation, PGC-1 α – peroxisome proliferator-activated receptor γ coactivator 1 α , RC – respiratory chain complex, SD – standard deviation.

ous system (Tabrizi et al., 1999; Powers et al., 2011). Ectodermal tissue, such as buccal swabs obtained by totally non-invasive sampling, might be more useful. It has already been shown to be a useful sample source for markers in other neuropsychiatric disorders (Goldenthal et al., 2015). Additionally, the dipstick immunocapture assay (DIA) has been successfully used to screen RC deficiency (Rodinová et al., 2014).

The aim of this study was to verify whether the amount of RC I and IV was altered in buccal epithelial cells of Huntington's disease patients.

Material and Methods

Subjects

A group of 34 patients (19 females and 15 males) with genetically proven HD (mean age 50.9 ± 14 years \pm SD, mean CAG triplet count 44 repetitions ranging from 40 to 53, mean disease duration 7.8 ± 3 year \pm SD) and a group of 22 healthy volunteers (11 females and 11 males, mean age 45.4 ± 13 years \pm SD) were analysed. Informed consent was obtained prior to taking samples, and the study was approved by the Ethical Committee of the General University Hospital in Prague. Age and gender did not differ between groups (Mann-Whitney U-test $P = 0.275$, Fisher's exact test $P = 0.786$, respectively).

In the HD group, 10 patients inherited the disease from the mother, nine inherited the disease from the father; in others the inheritance was unknown. From this group 13 patients were diagnosed with progressive weight loss (cachectic subgroup) and treated by nutritional intervention with high-calorie food supplements. At the time of sample collection, the cachectic subgroup had a mean BMI 20.6 ± 3 , while the other patients had a mean BMI 26.3 ± 4 . These subgroups did not differ in age or gender (Mann-Whitney U-test $P = 0.972$, Fisher's exact test $P = 1$, respectively).

Analysis

Buccal swabs were collected using Sterile OmniSwab (Whatman, St. Louis, MO) and within two hours stored at -80 °C. Samples were then processed using Complex I and Complex IV Human Protein Quantity Dipstick Assays (ab109722, ab109877, Abcam Mitosciences, Cambridge, UK): swabs were incubated in 500 μ l Buffer A on ice, lysed cell suspension was centrifuged for 20 min at 15,000 g and 4 °C, 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) was added to the supernatant. The total protein amount in the resulting solution was measured using Lowry protein assay (Lowry et al., 1951). An internal standard for each measurement series had been created by mixing nine lysed cell supernatants of healthy control subjects and the total protein amount was also measured using the Lowry protein assay.

A volume of each sample containing 200 μ g or 100 μ g of total protein amount was mixed with 25 μ l of Buffer B and transferred to a microplate well containing dried gold-conjugated Complex I or Complex IV antibody,

respectively. The same procedure was done with the internal standard. After antibody rehydration, the dipstick was added to the microplate well and the sample was allowed to wick onto it. Then, 30 μ l of washing Buffer C was added to the well and allowed to wick onto the dipstick to improve contrast. The dipstick was then dried and scanned, and the signal intensity of Complex I or Complex IV was measured using Quantity One 1-D Analysis Software (BIO-RAD Laboratories, Hercules, CA). A sample of each patient was analysed twice.

Statistical analysis

Clinical data were stored at REDCap for data collection and management (Harris et al., 2009) and then analysed using Python (version 2.7.12) with statistical libraries NumPy (version 1.11.0), SciPy (version 0.17.0), Pandas (0.19.2), matplotlib (version 1.5.1) and seaborn (version 0.7.1) (Oliphant 2007).

RC I and RC IV amounts and RC I/RC IV ratio in patient and control groups were compared using Mann-Whitney U-test, as in cachectic and non-cachectic patient subgroups. Pearson's correlation coefficient was calculated for correlation of RC I, RC IV and RC I/RC IV ratio with the age of patient and control groups and with CAG repeats in the patient group. The patient's effect of dependence of RC I, RC IV and RC I/RC IV ratio on age was analysed using the ordinary least squares regression model with interaction.

In all statistical analyses, the significance level 0.05 was used; P values were corrected using the Bonferroni method for family-wise error (FWE).

Results and Discussion

The patient and control groups did not show any difference in RC I, RC IV, or RC I/RC IV ratio. The RC I protein quantity was correlated with age in the patient group (Fig. 1, Pearson's $r = 0.427$); however, this correlation did not pass correction for multiple comparisons ($P = 0.026$, FWE- $P = 0.156$); other correlations for RC I, RC IV, RC I/IV ratio with age in both patients and controls and with CAG repeats in patients were not significant. The effect of the disease on RC-age dependence was not statistically significant.

There seemed to be a trend towards a difference between cachectic and non-cachectic patient subgroups; however, the data was not sufficient to prove that (Fig. 2, $P = 0.076$, FWE- $P = 0.228$). The nutrition status is likely to play an important role in regulating the expression of RC complexes. It was shown that activities of RC I in the group of females with anorexia nervosa was significantly increased in comparison with age-related controls in isolated platelets (Böhm et al., 2007). Furthermore, the ratio of complex I activity to the activity of control enzyme CS was significantly higher in the group of females with the restrictive type of anorexia nervosa characterized as a chronic food restriction than in the group of females with the purgative type of anorexia nervosa (Böhm et al., 2007). The abundance of NDUFB8

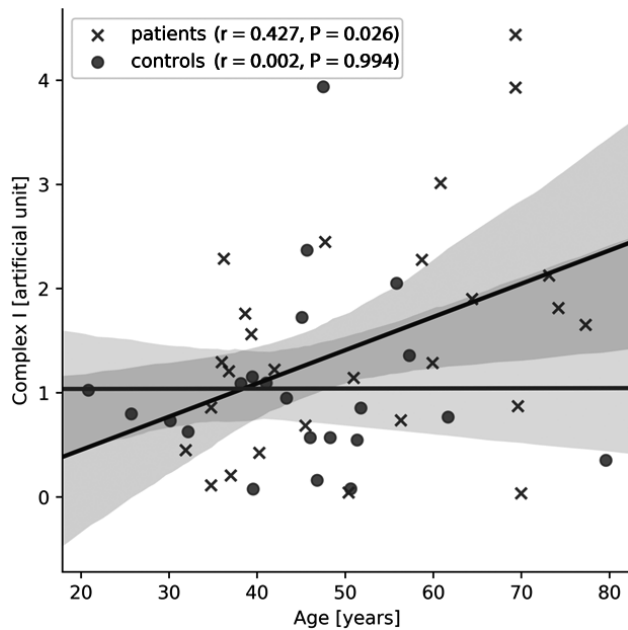


Fig. 1. Correlation between respiratory chain Complex I quantity and age in HD patients and in controls. Patients: N = 34, controls: N = 22. Complex I quantity in buccal epithelial cells was measured using Human Protein Quantity Dipstick Assay (ab109722, Abcam MitoSciences).

protein, a marker of RC I, was also significantly greater in the liver of mice on 30% caloric restriction than in the mice fed ad libitum (Kim et al., 2015).

There seemed to be a much stronger RC I dependence on age in the HD patient group than in the control group. In other neurodegenerative diseases such as Parkinson's or Alzheimer's diseases, it has been well shown that RC I deficiency correlates with mitochondrial dysfunction and RC I misassembly (Giachin et al., 2016). Most of the studies measured RC I activity and not RC I protein quantity; however, in one quantitative proteomic study, the RC I protein quantity was upregulated in a mouse model of Alzheimer's disease in addition to the RC I activity deficit (Rhein et al., 2009). We may speculate that the up- or down-regulation of RC I expression may serve as a protective mechanism. It was shown that the expression of Ndi1 (NADH dehydrogenase internal 1) in flies, acting as a non-proton translocating NADH dehydrogenase/ubiquinone oxidoreductase in the mitochondrial matrix, resulted in increased markers of complex I-like activity, extended longevity, and reduced ROS levels in a tissue-specific manner (Bahadorani et al., 2010; Sanz et al., 2010; Hur et al., 2013).

Conclusions: The relationships of RC I and RC IV with clinical data are not strong enough for individual analysis of each patient, but buccal epithelial swabs in combination with RC I amount dipstick immunoassay seem to be useful for group studies. With its non-invasive approach, it could be proved useful in epidemiological studies.

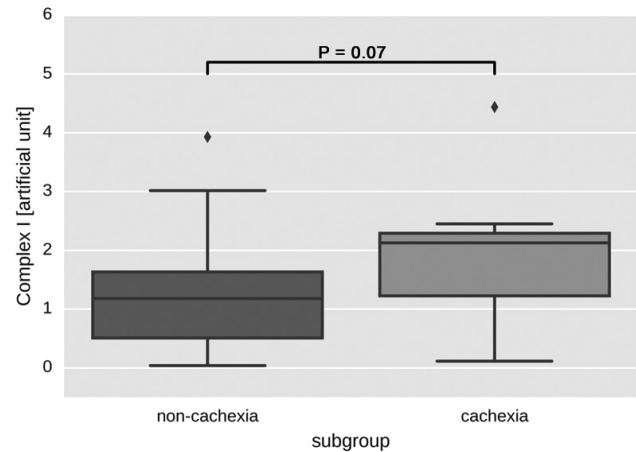


Fig. 2. Respiratory chain Complex I quantity in cachectic and non-cachectic subgroups of HD patients. Cachectic patients: N = 13, non-cachectic patients: N = 11. Complex I quantity in buccal epithelial cells was measured using Human Protein Quantity Dipstick Assay (ab109722, Abcam MitoSciences).

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