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Cerebrospinal fluid levels of coenzyme Q10 are reduced in multiple system atrophy

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ABSTRACT

Introduction: The finding of mutations of the *COQ2* gene and reduced coenzyme Q10 levels in the cerebellum in multiple system atrophy (MSA) suggest that coenzyme Q10 is relevant to MSA pathophysiology. Two recent studies have reported reduced coenzyme Q10 levels in plasma and serum (respectively) of MSA patients compared to Parkinson's disease and/or control subjects, but with largely overlapping values, limited comparison with other parkinsonisms, or dependence on cholesterol levels. We hypothesized that cerebrospinal fluid (CSF) is reliable to assess reductions in coenzyme Q10 as a candidate biomarker of MSA.

Methods: In this preliminary cross-sectional study we assessed CSF coenzyme Q10 levels in 20 patients with MSA from the multicenter Catalan MSA Registry and of 15 PD patients, 10 patients with progressive supranuclear palsy (PSP), and 15 control subjects from the Movement Disorders Unit Biosample Collection of Hospital Clinic de Barcelona. A specific ELISA kit was used to determine CSF coenzyme Q10 levels. CSF coenzyme Q10 levels were compared in MSA vs. the other groups globally, pair-wise, and by binary logistic regression models adjusted for age, sex, disease severity, disease duration, and dopaminergic treatment.

Results: CSF coenzyme Q10 levels were significantly lower in MSA than in other groups in global and pair-wise comparisons, as well as in multivariate regression models. Receiver operating characteristic curve analyses yielded significant areas under the curve for MSA vs. PD, PSP and controls.

Conclusions: These findings support coenzyme Q10 relevance in MSA. Low CSF coenzyme Q10 levels deserve further consideration as a biomarker of MSA.

1. Introduction

Multiple system atrophy (MSA) is an incurable and rapidly progressive neurodegenerative condition, clinically grouped with atypical parkinsonisms and neuropathologically classified as a synucleinopathy [1]. From a clinical standpoint, MSA has parkinsonism (parkinsonian variant: MSAp) or ataxia (cerebellar variant: MSAc), plus dysautonomia (which is mandatory for the clinical diagnosis) as its core features [1]. Currently MSA can only be confirmed neuropathologically, with the clinico-pathological mismatch being particularly due to misdiagnose as Parkinson's disease (PD) or progressive supranuclear palsy (PSP), another atypical parkinsonism, but with underlying tauopathy instead of synucleinopathy [2,3]. In view of this and the foreseeable emergence of disease-specific treatments, many efforts are underway to develop reliable biomarkers for these conditions.

The finding of mutations of the *COQ2* gene [4] and reduced coenzyme Q10 in the cerebellum in MSA [5,6], have led to suggest that coenzyme Q10 is relevant to the pathophysiology of MSA

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(perhaps through mitochondrial dysfunction and oxidation of alpha-synuclein) [7] and that it might be both a biomarker and a potential therapeutic target in this condition. Two recent studies have respectively shown reduced coenzyme Q10 levels in serum [8] and plasma [9] of MSA patients. However, in both studies the overlap among groups was remarkable, in the plasma study MSA was compared only to controls, and in the serum study differences were significant only after controlling for cholesterol levels, a known confounder of blood coenzyme Q10 levels.

One of the most widely explored sources of biomarkers of neurodegeneration is cerebrospinal fluid (CSF), after its close relationship with brain tissue and the fact that, if properly collected, it is less likely of being influenced by systemic metabolites, as opposed to blood. Moreover, albeit its collection is invasive, it can be routinely, easily and safely performed [10].

For all these reasons we hypothesized that CSF is reliable to assess coenzyme Q10, and that CSF coenzyme Q10 levels are lowered in MSA vs. other neurodegenerative parkinsonisms and controls.

2. Methods

2.1. Design

This is a pilot hypothesis-driven cross-sectional convenience study based on samples from the multicenter Catalan MSA Registry (CMSAR) and the Movement Disorders Biosample Collection of Hospital Clinic de Barcelona (MDBC-HCB). In short, the CMSAR is a multicenter initiative in which movement disorders specialists across Catalonia identify possible or probable MSA patients and offer them to participate in the registry and to donate biosamples (blood, urine, fibroblasts, CSF) at Hospital Clinic de Barcelona. The MDBC-HCB is a single centre biorepository consisting of several biosamples such as CSF of patients with degenerative parkinsonisms, including PD and PSP patients. Both the CMSAR and the MDBC-HCB along with the present CSF biomarker study have received approval from the competing Institutional Review Board, and all participants provided their written informed consent.

2.2. Participants

Sixty participants were studied: 20 sporadic MSA cases from the CMSAR clinically diagnosed according to currently accepted criteria [11] (10 classified as MSAp and 10 as MSAc; 13 as probable and 7 as possible MSA), and 40 additional subjects having contributed to the MDBC-HCB, and consisting of: 15 non-demented PD patients (all with a clinically definite diagnosis according to the United Kingdom Parkinson's Disease Society Brain Bank criteria [12]); 10 patients with clinically probable PSP according to the NINDS-SPSP criteria [13]; and 15 controls who after comprehensive clinical history and examination as well as brain MRI without remarkable changes were judged not to have any known neurodegenerative disease. These control subjects donated CSF during their admission for knee replacement surgery with intradural anaesthesia. Part of the PD, PSP and control subjects have been reported in previous CSF studies [14,15].

Hoehn & Yahr stage at the time of inclusion was recorded [16] for MSA, PD and PSP participants. Additionally, the scores of the unified MSA rating scale (UMSARS) [17] and the motor section of the unified PD rating scale (UPDRS-III) [18] were available for MSA and PD patients, respectively. Mini mental state examination [19] and Mattis dementia rating scale (MDRS-2) [20] were used for cognitive assessment. Levodopa equivalent daily dose (LEDD) was calculated [21].

2.3. CSF collection, storage and analysis

All patients underwent lumbar puncture (LP) in L3-L4 space, using a 22G needle, between 8 and 10 a.m., after overnight fasting and under off-medication. The first 2 mL of CSF were used for routine studies. The following 10 mL of CSF were collected in polypropylene tubes and immediately centrifuged for 10 min at 4,000*g* and 4 °C, and stored at −80 °C in 300 μL polypropylene aliquots until analyses. After LP, patients stayed in bed for at least 2 h and were advised to increase water intake. To determine CSF coenzyme Q10 levels we used a commercially available specific ELISA technique (Human CoQ10 ELISA Kit; MBS701260; MyBioSource; San Diego, CA, USA). All CSF samples were analyzed in duplicate using two ELISA kits with the same batch number, with 11 samples being tested in both experiments to control for inter-assay variability.

2.4. Statistical analyses

All data were analyzed using SPSS 20.0 (IBM, New York, USA). No formal statistical power calculations were carried out due to: (a) the pilot and exploratory nature of the study, (b) the rarity of the disease and the consequent unfeasibility to recruit large samples, and (c) the paucity of previous data on coenzyme Q10 in MSA, in general, and using CSF, in particular. Yet, considering both previous studies of coenzyme Q10 using peripheral blood with sample sizes in the range of ours [8,9] and prior CSF studies of other biomarkers with significant findings using similar sample sizes [14,15,22], the available cohort was deemed likely to allow for detecting relevant associations. Qualitative variables are presented as relative and absolute frequencies and compared with Fisher's exact test. Quantitative variables are reported as medians and interquartile ranges, and compared across all groups by means of Kruskal-Wallis followed by pairwise Mann-Whitney's U tests between MSA and each of the other groups (conditioned to only when the overall group test was significant), with post-hoc Bonferroni's correction for multiplicity (all reported pairwise p-values are Bonferroni-corrected). Linear correlations were explored with Spearman correlation. Binary logistic regression models allowed for further testing of CSF coenzyme Q10 levels as a continuous quantitative predictor (expressed in ng/mL) with adjust for potential qualitative or quantitative modifiers (age, sex, disease duration, disease severity as per motor scales, LEDD). In the statistical analyses plan, at first several models were run separately, each one adjusted for part of the aforementioned potential modifiers, since the cohort size made it less reliable introducing all the potential modifiers at once in a single model, and as some of these variables were not applicable to the control group (disease duration, motor scales, LEDD). However, one such model pooling all the variables together was also run in the end, for exploratory purposes and as additional data. The results of these binary logistic regression models are presented as odds ratios (OR) and the respective 95% confidence intervals (95%CIs), with OR>1 indicating an increase in the outcome risk per each unit increase in the tested variable, with the opposite applying for OR<1. Finally, CSF coenzyme Q10 levels were additionally tested as a potential MSA biomarker by means of receiver operating characteristic (ROC) analyses, where areas under the curve (AUCs) and 95%CIs greater than 0.5 indicate significant discriminant ability. All analyses were two-tailed, with p-threshold set at $\leq$0.05.

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