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Cyclophilin D regulates lifespan and protein expression of aging markers in the brain of mice

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ABSTRACT

Cyclophilin D (cypD) modulates the properties of the permeability transition pore, a phenomenon implicated in the manifestation of many diseases including aging. Here, we examined the effects of partial or complete deletion of cypD on i) lifespan, ii) forebrain protein expression of 18 aging markers as well as regional expression of GFAP, mGluR1, and alpha-synuclein, and iii) behaviour of aged (>24 month) male and female mice. Both male and female cypD heterozygous but not KO mice exhibited increased lifespans compared to WT littermates, associated with alterations in the protein expression of some markers, albeit without exhibiting changes in behaviour.

1. Introduction

Cyclophilin D (cypD) is a mitochondrially localized peptidyl-prolyl cis-trans isomerase (Johnson et al., 1999) and a binding target of the immunosuppressant cyclosporin A (Davis et al., 2010; Halestrap and Davidson, 1990). Both physiological and pathological functions of this protein stem from its isomerase domain (Baines et al., 2005), modulating the mitochondrial permeability transition pore (PTP) (Bernardi et al., 2015a). The PTP is a non-selective high-conductance channel which allows the flux of water and other molecules up to 1500 Da across the inner mitochondrial membrane (Azzolin et al., 2010). The physiological role of cypD and the PTP is to link mitochondrial ATP production with cellular functional demand through Ca^{2+} (Korge et al., 2011; Barsukova et al., 2011), reviewed in Elrod and Molkentin (2013). The contribution of PTP to pathology has been much more intensely investigated, aided greatly by the availability of cypD knock-out (KO) mice (Baines et al., 2005; Basso et al., 2005; Nakagawa et al., 2005; Schinzel et al., 2005). A vast body of work has led to the understanding that cypD is a critical determinant of a number of pathologies (Bonora et al., 2015), and thus, a drug target (Waldmeier et al., 2003). Indeed, cypD deficient mice overexpressing mutant amyloid precursor protein (mAPP) exhibited less Ca^{2+}-induced mitochondrial swelling, increased mitochondrial Ca^{2+} uptake capacity, preserved mitochondrial respiratory function and improved spatial learning/memory, even in old age (22–24 months of age) (Du et al., 2011). Accordingly, cypD deficiency attenuated mitochondrial and neuronal perturbation in addition to preserving learning and memory in a mouse model of Alzheimer's disease (Du et al., 2008), examined at 12 months of age. Furthermore, the genetic ablation of cypD delayed disease onset, and extended the lifespan of mutant α-synuclein mice (Martin et al., 2014). Importantly, not just in the mAPP overexpressing mice (Du et al., 2008) but also cypD deficiency alone improved spatial learning and memory.

On the other hand, mice lacking cypD exhibited an enhancement of anxiety, avoidance behaviour was facilitated, and adult-onset obesity was prominently evident from the 10 month of age, which was not dependent on increased food and/or water intake (Luvisetto et al., 2008). cypD KO mice also exhibited substantially greater cardiac hypertrophy, fibrosis, and reduction in myocardial function in response to pressure overload stimulation than WT mice (Elrod et al., 2010). Along these lines, CaMKIIδc overexpression in cypD KO mice resulted in heart failure characterized by a loss of left ventricular function and significant mortality (Elrod et al., 2010). Yet, clinical trials have shown that

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administration of the cyclophilin D inhibitor, cyclosporin A, was protective to patients immediately after myocardial infarction, when applied during the revascularization phase (Piot et al., 2008).

Apart from their contribution to the aetiology of diseases, mitochondria have also been considered to be involved in aging (Crompton, 2004; Toman and Fiskum, 2011). Relevant to this, aging studies in the human and murine brain resulted in the identification of "aging markers" that concern gene expression and DNA damage. Specifically, from post-mortem samples of the frontal pole of 30 individuals ranging in age from 26 to 106, RNA was harvested and analysed using Affymetrix gene chips (Lu et al., 2004) and age-related genes were identified by performing statistical group comparison of frontal cortical samples from individuals ≤42 and ≥73 years old; it was found that about 4% of the approximately 11,000 genes analysed were significantly changed (1.5-fold or more) (Lu et al., 2004). Likewise, the effects of aging on gene expression in the brain of mice have also been examined (Jiang et al., 2001). Statistically significant differences in gene expression in the hypothalamus and cortex of young (2 months old) and aged (22 months old) mice have been found by using high-density oligonucleotide arrays, and a number of key genes involved in neuronal structure and signalling were differentially expressed (Jiang et al., 2001). A similar study using oligonucleotide arrays representing 6347 genes determined the gene-expression profile of the aging neocortex and cerebellum in mice (Lee et al., 2000). There, it was found that aging resulted in a gene-expression profile indicative of an inflammatory response, oxidative stress and reduced neurotrophic support in both mouse brain regions (Lee et al., 2000).

Mindful of the connection of cypD to PTP and that of the latter to aging, we examined the contribution of partial or complete deletion of cypD in protein expression of aging markers in the mouse brain. Given that in the human aging brain, gene expression changes are sexually dimorphic (Berchtold et al., 2008), we examined both male and female mice. In the study where human frontal cortex aging markers were sought (Lu et al., 2004), 466 genes exhibited very significant alterations. This gene pool was subdivided in 11 "functions", namely, synaptic functions, vesicular transport, neuronal survival, protein turnover, amino acid modification, mitochondrial functions, stress response, inflammation, myelination/lipid metabolism, transcription, and hormonal functions. From these 466 genes, we selected 15 proteins on the basis of four criteria: i) there are well-characterized mouse homologues, ii) they are distributed among most of the 11 subdivided gene pools, iii) they show a large up- or down regulation compared to all members of the 466 genes, and iv) there are verified antibodies available, which are suitable for both Western blotting and immunohistochemistry for the expressed proteins in mice. These proteins were: ATP6V1H, Calbindin, calcineurin B, Cdk5, GFAP, HIF1α, Leptin Receptor, MEK4, mGluR1, PKC-γ, Synapsin, Sortilin and VAMP1. Additionally, we examined the expression of α-synuclein, a marker which is central to disease-progression in Alzheimer's disease as well as MnSOD as a mitochondrial marker. GAPDH and β-actin were used as house-keeping genes. Some of the above aging markers were further selected for quantitative evaluation of immunohistochemical sections of the motor cortex, hippocampal and thalamic regions of the mice. Finally, in addition to examining protein expression, we also compared the lifespan of the mice groups, and evaluated their behaviour at >24 months of age.

2. Results

2.1. The effect of partial or complete deletion of cypD in the lifespan of male and female mice

As shown in Fig. 1A, the survivals of male WT (n = 514), HT (n = 147) and KO (n = 644), and in panel 1B female WT (n = 322), HT (n = 145) and KO (n = 358) were recorded until the time of their natural death, and plotted using Kaplan-Meier analysis. By applying a *post-hoc* with a Holm-Sidak test for pairwise multiple comparisons, it is

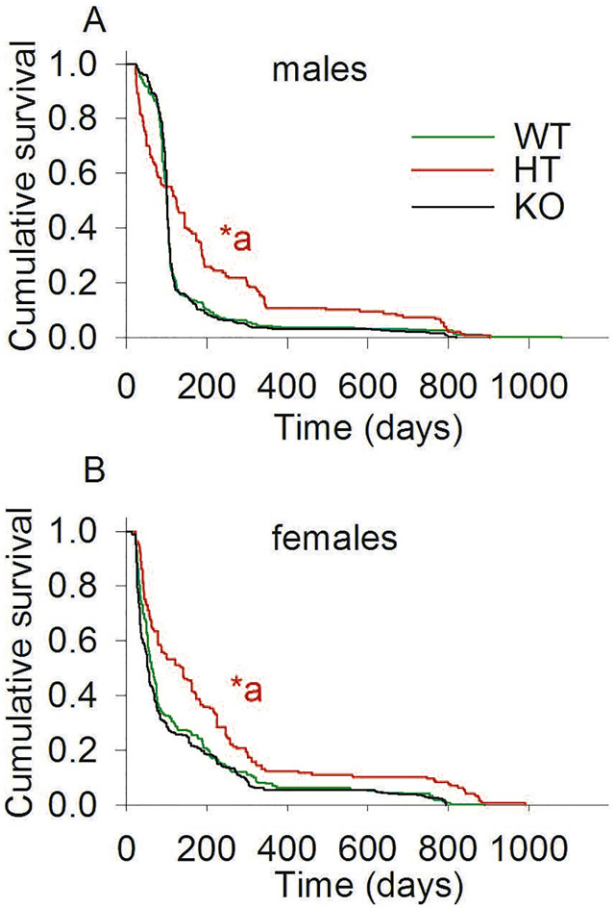

Fig. 1. The effect of partial or complete deletion of cypD in the lifespan of male and female WT, cypD heterozygous and cypD KO mice. Kaplan-Meier plots were calculated from lifetime data of WT (green), HT (red) and KO (black) CypD mice. *a < 0.001, (HT vs WT or KO, either gender) using a *post-hoc* with a Holm-Sidak test for pairwise multiple comparison.

apparent that both male and female HT mice exhibited longer lifespan ($p < 0.001$) than both WT and KO mice. No statistical significant difference was observed between WT and KO mice of either gender. Some of these mice have undergone behavioural evaluation at the age of 24 months (see Section 2.4). Interestingly though, male HT mice exhibited an early lethality compared to KO and WT mice. Gross pathological examination of the deceased mice did not show any major abnormalities, though the exact cause of death was not estimated. The reason(s) for the early lethality of male HT mice was not investigated further.

2.2. The effect of partial or complete deletion of cypD in the expression of aging markers in the forebrain of mice

As shown in Fig. 2 for male and Fig. 3 for female, forebrain homogenates from 5 WT, 5 HT and 5 KO mice (all >24 months of age) were probed for the expression of the following proteins: cypD, β-actin, GAPDH, α-synuclein, ATP6V1H, calbindin, calcineurin B, Cdk5, GFAP, HIF1α, leptin receptor, MEK4, mGluR1, MnSOD, PKC-γ, synapsin, sortilin and VAMP1. On the left side of the figures, scanned images of the Western blots are shown. The densitometric analysis of the bands (assigning an O.D. of 1 for the averaged bands obtained from the WT mice, black bars) is shown as graphs, plotted as means with bars representing the standard error of the mean, in the panels to the right. Statistical significance was determined comparing the 3 groups (WT, HT, KO) by ANOVA on Ranks followed by one-way ANOVA and Tukey's test *post-hoc* analysis. Data with $p < 0.05$ were considered significant. All antibodies