Department of Child and Adolescent Psychiatry and Psychotherapy
Translational Molecular Psychiatry

ELUCIDATING THE FUNCTIONAL EFFECTS OF OMEGA-3 FATTY ACIDS AS A TREATMENT IN ADHD AGAINST INFLAMMATION AND OXIDATIVE STRESS

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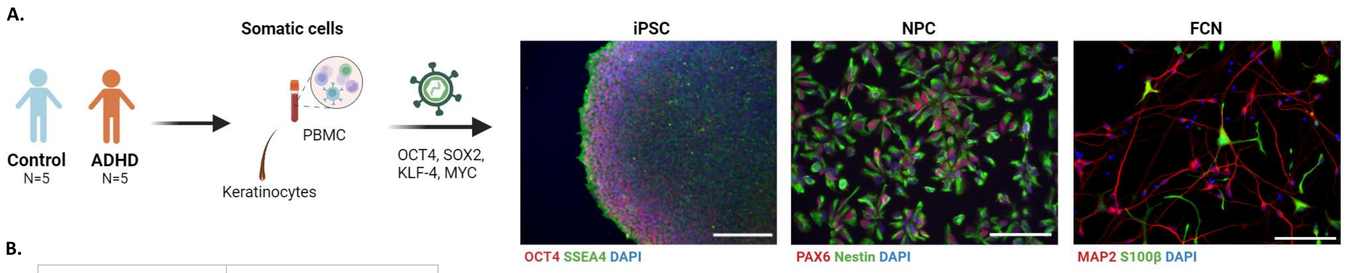
BACKGROUND

Attention-deficit hyperactivity disorder (ADHD) is the most frequently reported neurodevelopmental disorder, with a worldwide prevalence of ca. 5%, affecting children and adolescents. Inflammation and oxidative stress may play a crucial role in ADHD, indicated by altered serum levels of IL-6 and TNFα cytokines as well as increased reactive oxygen species discovered in ADHD children [1,2]. Therefore, the non-pharmacological treatment of omega-3 (ω-3) polyunsaturated fatty acid (PUFA) components, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), display potential candidates, as they take part in several biological processes, including the attenuation of inflammation and oxidative stress. However, the underlying molecular mechanisms of ω-3 PUFAs involved in ADHD remains unknown.

METHODS



B.

Control	ADHD
K001 i6	MR001 x15
K005 z13	MR010 c3
K011 c10	MR013 c3
K015 c1	MR014 c12
K013 c39	MR030 c2

Figure 1. Recruitment and induced pluripotent stem cell (iPSC)-derived neural precursor cells (NPC) and forebrain cortical neurons (FCN) generation. A. Scheme displays the reprogramming of Peripheral Blood Mononuclear Cells (PBMCs) and hair-derived keratinocytes to iPSCs using Sendai virus transduction; and further generation of NPCs and FCNs (created with BioRender). B. Table lists generated cell lines from ADHD patients (N=5, 4 male) and controls (N=5, 4 male) (mean age 13 ± 3) recruited at the KJPP, UZH [3-5]. Male=black; Female: red.

AIM OF THE STUDY

We aim to investigate the effects of ω-3 PUFAs on pro-inflammatory cytokine release and oxidative stress in human induced pluripotent stem cell (iPSC)- derived forebrain cortical neurons (FCNs) from ADHD patients and healthy individuals as control.

RESULTS

OXIDATIVE STRESS

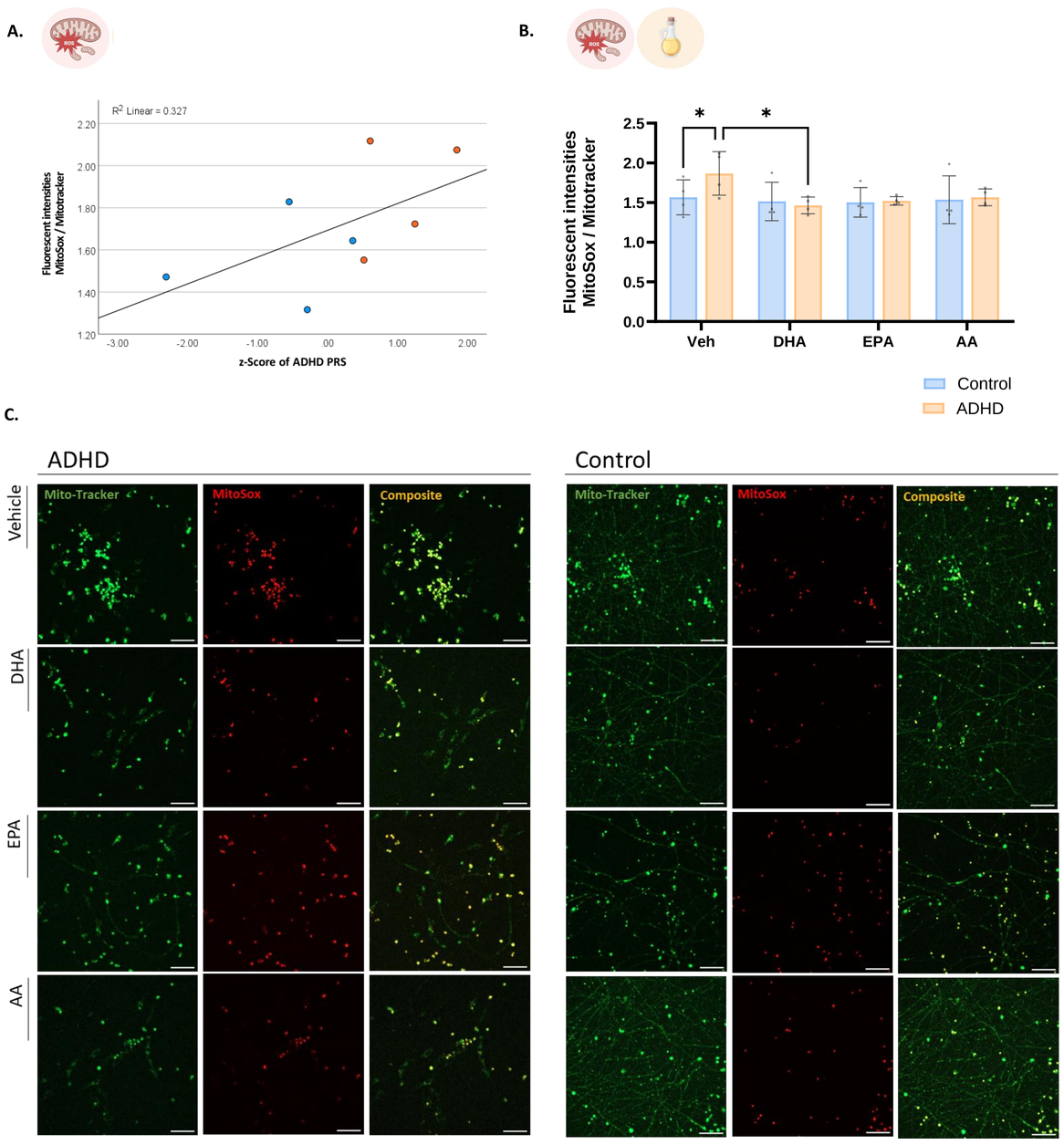


Figure 2. Investigation of mitochondrial derived oxidative stress in FCNs. A. Correlation analysis displaying the z-Score of ADHD PRS against the fluorescent intensities of MitoSox marking superoxides normalized by Mitotracker stainings in FCNs of ADHD and controls. B. Bar chart represent mean value ± SD of fluorescent intensities of MitoSox normalized by Mitotracker in controls (blue; N=4) and ADHD (orange; N=4) after ω-3 PUFA treatment DHA (10μM) and EPA (10μM) and ω-6 PUFA treatment Arachidonic acid (AA; 10μM) for 48h (Ordinary Two-way ANOVA with Tukey's post-hoc test; p-value * 0.01 ≤ p < 0.05). C. Representative images Of MitoSox and Mitotracker stainings in ADHD (left) and control (right) FCNs after ω-3/6 PUFA treatments (10μM)

INFLAMMATION

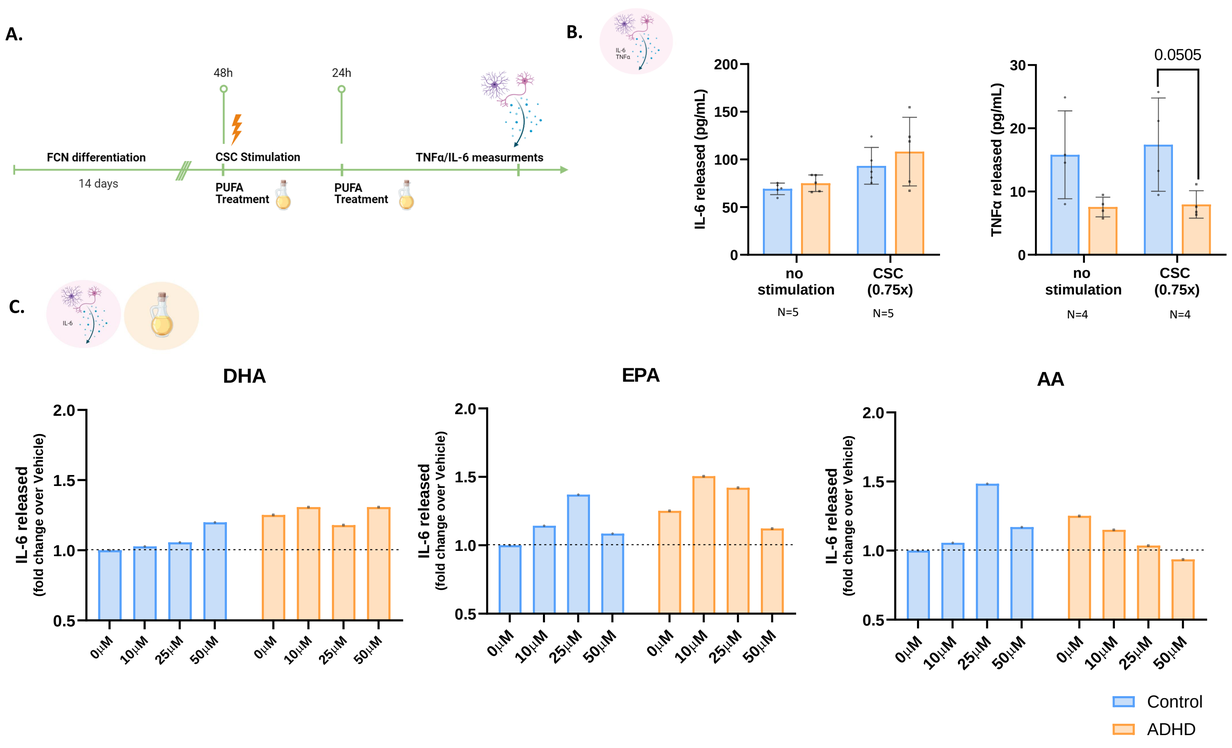


Figure 3. Pro-inflammatory cytokine release IL-6 and TNFα in FCNs. A. Experimental outline displays the cellular stimulation with a Cell Stimulation Cocktail (CSC) of FCNs 48 hours prior to pro-inflammatory cytokine IL-6 and TNFα release measurements. PUFA treatment time points were conducted at 48h and 24h prior to measurements. FCNs were measured after 14 days differentiation (created with BioRender). B. Bar charts represent mean ± SD of IL-6 release (left) and TNFα release (right) with and without CSC (0.75x) stimulation of ADHD (orange) and control (blue) FCNs. C. Graphs represent mean value of IL-6 released fold change over control vehicle after CSC stimulation as well as DHA (0-50μM), EPA (0-50μM) and AA (0-50μM) treatment in FCNs from controls and ADHD groups.

MITOCHONDRIAL DNA COPY NUMBER VARIATION

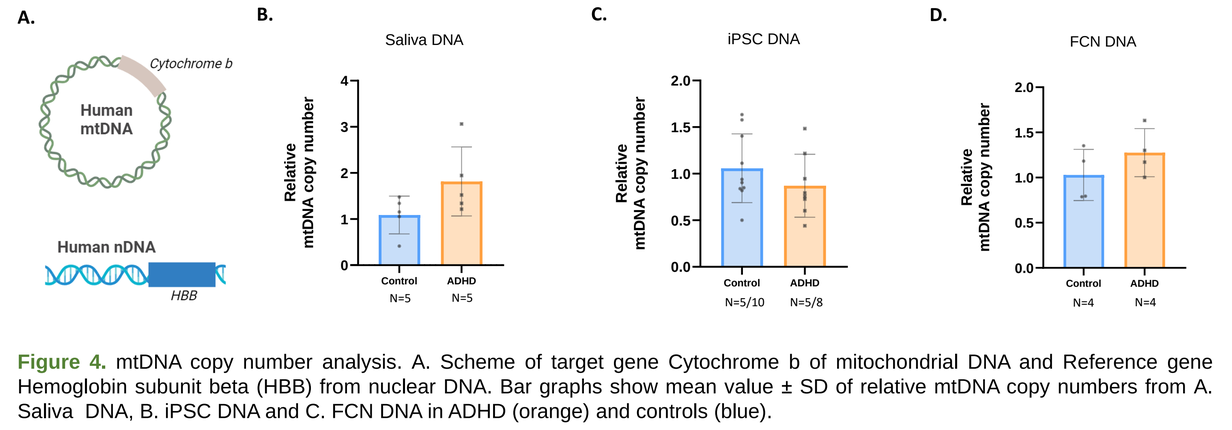


Figure 4. mtDNA copy number analysis. A. Scheme of target gene Cytochrome b of mitochondrial DNA and Reference gene Hemoglobin subunit beta (HBB) from nuclear DNA. Bar graphs show mean value ± SD of relative mtDNA copy numbers from A. Saliva DNA, B. iPSC DNA and C. FCN DNA in ADHD (orange) and controls (blue).

CONCLUSION

- ADHD cell lines display significantly higher oxidative stress derived from mitochondria, implying mitochondrial disturbances in ADHD. PUFA allow the reduction of superoxide levels in ADHD, diminishing alterations seen compared to controls. Thus, PUFA show beneficial effects against oxidative stress in ADHD.
- Tendencies of increased mtDNA copy number in saliva and FCNs may suggest mitochondrial impairments in differentiated cells, as iPSCs don't display these alterations. Therefore, mitochondrial disturbances may occur during development.
- ADHD FCNs display elevated IL-6 secretion and declined TNFα levels. Moreover, ADHD cells tend to release more IL-6 after inflammatory stimulation suggesting alterations in pro-inflammatory response regulation.
- DHA, EPA and AA treatment may slightly alter IL-6 release in a dose-dependent manner after inflammatory stimulation of ADHD and control FCNs.

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