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Determination of neuroprotective effects of medium chain fatty acids and their derivatives on mutant huntingtin aggregates, oxidative stress and ATP levels in HD150Q cell line model of Huntington's disease

Rajubhai Dabhi¹, Ragi Mehta¹, Jayshree Paneri², Dhruvi Kakadiya¹, Anjali Shah¹, Sushmita Singh¹ & Ravi Vijayvargia^{1*}

¹Neurobiology and Molecular Diagnostics Lab., Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India

²Department of Biotechnology, Mohanlal Sukhadia University, Udaipur, Rajasthan, India

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Huntington's disease (HD) is a rare autosomal dominant genetic disorder resulting from expansion of polymorphic CAG repeats in the exon 1 of huntingtin gene that translates into elongated polyglutamine (polyQ) tract in huntingtin protein (HTT). PolyQ expansion alters HTT structure resulting in abnormal protein-protein interactions, aggregation, mitochondrial dysfunction, oxidative and endoplasmic reticulum stress, inflammation and altered gene expression leading to neuronal cell death. HD symptoms involve chorea, dementia, behavioural and psychological problems and currently there is no cure highlighting the need for novel therapeutic interventions. Several fatty acids have been reported to have protective effects in neurological disorders including Alzheimer's disease, Parkinson's disease and epilepsy. However, their effects in HD is largely unexplored. Neurodegenerative diseases share several common pathways and thus it is likely that a combination of selected fatty acids show neuroprotective effects in HD. This study utilized a cell line model of HD expressing inducible mutant huntingtin fragment with 150 polyQ repeats (HD150Q) to investigate neuroprotective effects of two medium chain fatty acids and one triglyceride. Significant reduction in mutant HTT aggregates and mitochondrial oxidative stress and restoration of ATP levels was observed upon treatment with Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate. Encouraging results in the cell line model opens avenues for investigating the underlying molecular mechanisms and validation in the animal models.

Keywords: Decanoic acid, 2-butyloctanoic acid, Glycerol triacetate, Huntington's disease, Mitochondrial dysfunction, Mutant huntingtin aggregates, Oxidative stress

Huntington's disease (HD) is a rare autosomal dominant neurodegenerative disorder, occurring with an incidence of 1 in 10,000 individuals and has no cure¹. The etiology of HD involves an abnormal expansion of CAG repeats in exon 1 of the huntingtin (*Htt*) gene that translates into expanded polyglutamine (poly Q) tract in mutant huntingtin (mHTT) protein resulting in alteration of HTT structure and facilitating abnormal protein-protein interaction and mHTT aggregation within neurons². These gain of function attributes of mHTT protein initiates a degenerative cascade, primarily affecting medium spiny striatal and cortical neurons, leading to dysfunction and subsequent neuronal loss, giving rise to neuromuscular manifestations such as involuntary movements, psychiatric symptoms, and cognitive decline³. Mutant huntingtin expression leads to dysregulated gene expression, mitochondrial dysfunction, endoplasmic reticulum (ER) stress,

oxidative stress, glutamate-mediated excitotoxicity, and cell death in a cell-autonomous manner^{4,5}. Furthermore, perturbations in ATP, a vital energy source for cellular processes, are observed in HD, contributing to compromised neuronal function⁶. Consequently, targeting the toxic effects of mHTT in neuronal context has been the primary focus of global research efforts.

Currently, HD lacks a definitive cure, and available treatments primarily focus on alleviating motor and psychological symptoms⁷. Fatty acids have emerged as potential therapeutic agents due to their multifaceted roles in neuronal health and function. Notably, omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) have demonstrated neuroprotective effects in various neurodegenerative disorders, including Alzheimer's and Parkinson's diseases⁸⁻¹³. While the neuroprotective role of PUFAs and MUFAs has received extensive investigation¹⁴⁻¹⁸, the involvement of saturated medium-chain fatty acids (MCFAs) in neurodegenerative processes has gained recent attention¹⁹.

*Correspondence:

E-mail: ravi.vijayvargia-biochem@msubaroda.ac.in

Decanoic acid (C10:0) and 2-butyloctanoic acid are specific saturated medium-chain fatty acids (MCFAs) that hold promise as neuroprotective agents in neurodegenerative diseases. Decanoic acid exhibits antiepileptic properties by inhibiting neuronal excitability and enhancing GABAergic neurotransmission²⁰. Additionally, it demonstrates neuroprotective effects in Alzheimer's disease models through the enhancement of mitochondrial function²¹. 2-butyloctanoic acid, a derivative of Decanoic acid, possesses anti-inflammatory properties, modulating microglial activation and reducing neuroinflammatory responses in models of Parkinson's and Alzheimer's diseases²². The precise mechanisms underlying their neuroprotective effects are still under investigation. Nonetheless, their ability to modulate neuronal excitability, improve mitochondrial function, and regulate neuroinflammatory responses underscores their potential therapeutic applications in neurodegenerative disorders. Furthermore, the specific effects of fatty acids and their derivatives on mHTT aggregates, oxidative stress, and ATP levels in HD are currently not well characterized.

This study aims to investigate the potential neuroprotective properties of fatty acids and their derivatives in HD by utilizing a cell line model of Huntington's disease. Given that fatty acids are obtained from diet and are generally safe to consume, the neuroprotective effect may lead to development of novel therapeutics for HD. Understanding the underlying molecular mechanisms implicated in the neuroprotective effects of fatty acids could pave the way for targeted interventions and contribute to the ultimate goal of alleviating the burden imposed by Huntington's disease.

Materials and Methods

Cell culture

The Neuro 2a cell line, expressing enhanced green fluorescent protein (e-GFP)-tagged truncated N-terminal huntingtin (Tn-HTT) with 150 polyglutamine (150Q) repeats under the regulation of an ecdysone-inducible promoter, was a kind gift from Prof. Nihar Ranjan Jana (National Brain Research Centre, Gurgaon, Haryana, India) and will be referred to as HD150Q. HD150Q cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Cytiva HyClone, USA), supplemented with 10% heat-inactivated fetal bovine serum (Cytiva HyClone, USA) and 1% (v/v) penicillin-streptomycin antibiotic mixture (Gibco, Invitrogen). The cells were maintained at 37°C in a humidified 5% CO₂ incubator. The HD150Q cell line

represents a stable dual transfectant, comprising two plasmids: pVgRXR (conferring Zeocin resistance) and pIND-HD. The selection of transgenic cells was accomplished by employing a concentration of 0.4 mg/mL Zeocin (InvivoGen) along with G-418 (Sigma-Aldrich). Notably, the HD150Q cells demonstrate expression of GFP-fused mutant huntingtin exclusively in the presence of Ponasterone A (Sigma-Aldrich).

Kits and reagents

Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate were procured from Sigma Aldrich. Anti-HTT antibody (Anti-HTT 4-19, N-Terminal) was procured from Coriell Institute for Medical Research (USA). Anti-Rabbit-HRP secondary antibody was obtained from Cell Signaling, USA. The ATP Determination Kit was acquired from Invitrogen, Thermo Fisher Scientific, USA, for the measurement of ATP levels. MitoSOX Red, a fluorescent dye for detecting mitochondrial superoxide, was purchased from Invitrogen, Thermo Fisher Scientific, USA.

Fluorescence microscopy

To visualize the aggregates of mutant huntingtin (mHTT) tagged with green fluorescent protein (GFP), HD150Q cells were plated in 6-well plates (NEST) at a density of 0.3×10^6 cells per well. The mHTT overexpression was induced by the addition of Ponasterone A (1 μ M) for indicated time points. To investigate the impact of Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate on mutant huntingtin aggregation, cells were treated with indicated concentrations of fatty acids or DMSO (vehicle control) for 48 h. Subsequently, the cells were stained with Hoechst 33342 (Invitrogen, Thermo Fisher Scientific, USA) and observed under an Eclipse Ti2-E inverted fluorescence microscope (Nikon, Japan). During the experiments, the gain, offset levels, and laser power of the detectors were meticulously calibrated and maintained at consistent settings. The quantification of mHTT puncta per field was performed manually and represented as average number of puncta per field.

Western blotting

HD150Q cells were seeded in 6-well plates at a density of 0.3×10^6 cells per well and subjected to the specified treatments for a designated duration. Following treatment, the cells were collected, rinsed with ice-cold 1X phosphate-buffered saline (PBS), and then suspended in lysis buffer (50 mM Tris-Cl, pH 8.0,

and 0.1% Triton X-100) supplemented with 1X protease inhibitor cocktail (Sigma-Aldrich, USA) and lysed by sonication using a bath sonicator, employing 10s ON/OFF cycles for a total of 3 rounds. The protein in resulting cell lysates was quantified using the Bradford assay (Bio-Rad, USA), and equal amounts of proteins were loaded and subjected to western blotting as described previously²³. Briefly, the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, USA) and blocked using a 5% blocking buffer (composed of 5% non-fat dried milk and 0.1% Tween-20 in TBS). The membrane was then incubated overnight at 4°C with anti-HTT antibody. After washing the membrane three times for 10 minutes each with 1X TBS containing 0.1% Tween-20 (TBST), the membrane was probed with an HRP-conjugated anti-rabbit secondary antibody (obtained from Cell Signaling, USA). Following another three washes with 1X TBST, chemiluminescent substrate was applied to the membrane, and the resulting blot bands were visualized using the ChemiDoc Touch Imaging System (Bio-Rad Laboratories, USA). The acquired blots were subsequently analyzed using Image Lab software (Bio-Rad Laboratories, USA). To determine the relative intensity of each protein's expression, ImageJ image processing software (developed by Rasband WS; NIH) was employed.

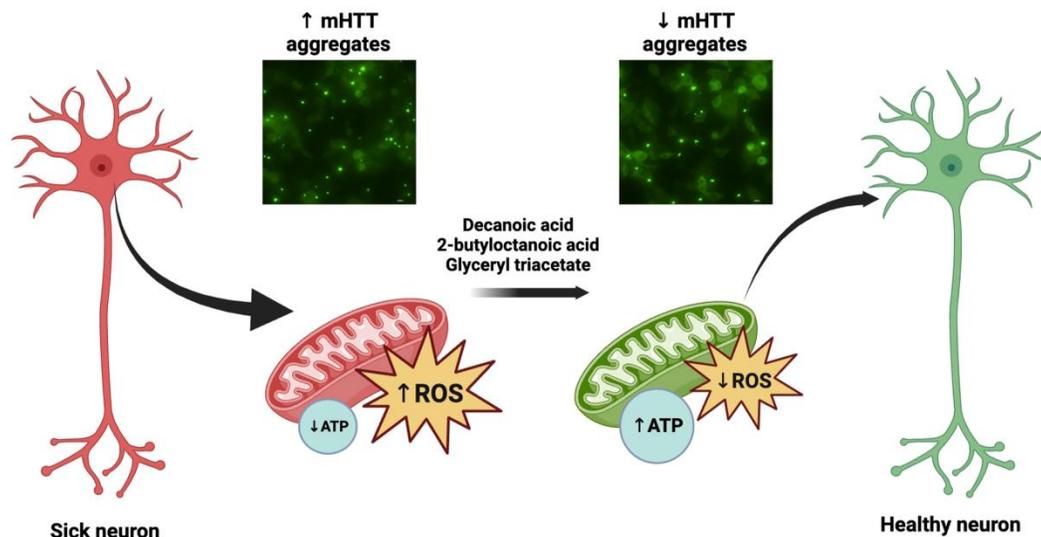
ATP levels

The impact of the administered fatty acid treatments on cellular ATP production was examined

using a luminescent-based ATP determination kit (Molecular Probes/Life Technologies, Canada), following the manufacturer's guidelines. HD150Q cells were treated with 1 μ M Ponasterone A and varying concentrations of the fatty acids for a duration of 24 h. Subsequently, the cells were washed with ice-cold 1X PBS and prepared for the assessment of the fatty acid treatments' influence on cellular ATP levels. The cells were lysed on ice using 0.1% Triton X-100, and a 10 μ L aliquot of the resulting cell lysate was diluted in 100 μ L of an ATP determination master mix comprising 25 mM Tricine buffer (pH 7.8), 5 mM MgSO₄, 0.5 mM D-luciferin, 1.25 μ g/mL firefly luciferase, 100 μ M EDTA, and 1 mM DTT. The luminescence intensity was measured utilizing a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments). The protein content was quantified using the Bradford assay, and the ATP concentrations were normalized with their respective protein concentrations.

Mitochondrial ROS determination

Mitochondrial Reactive Oxygen Species (ROS) levels were assessed through the utilization of MitoSOX Red staining at a concentration of 5 μ M. To initiate the experiment, HD150Q cells were seeded in 6-well plates at a density of 0.3×10^6 cells per well. The cells were then subjected to treatment with Ponasterone A and/or varying concentrations of the fatty acids, followed by staining with MitoSOX Red and Hoechst 33342 (Invitrogen, Thermo Fisher Scientific,



USA). Subsequently, the cells were observed and analyzed under an Eclipse Ti2-E inverted fluorescence microscope (Nikon, Japan). The mean fluorescence intensity was quantified using ImageJ software, and the obtained data were further processed and plotted using GraphPad Prism 6 software.

Statistical analysis

GraphPad Prism: version 6 software was primarily employed for conducting various statistical analyses and generating graphical representations of the obtained results. The experimental data, concerning the number of observations, is expressed as mean \pm SEM. In cases where a comparison was made between two groups with repeated measurements, the Student's unpaired *t*-test was utilized to determine the statistical significance of each group. For experiments involving more than two groups, the one-way analysis of variance (ANOVA) test was employed, followed by Dunnett's multiple comparison test to assess the level of significance. Each experiment was independently repeated a minimum of three times, and statistical significance was considered at a probability value of $P \leq 0.05$.

Results

Decanoic Acid, 2-Butyloctanoic Acid, and Glycerol Triacetate prevent mHTT Aggregation

In this study, Fluorescence microscopy was employed to visually examine and quantify the presence of GFP-tagged mutant huntingtin (mHTT) aggregates in the HD150Q cells. The primary objective was to assess the ability of Decanoic acid, 2-Butyloctanoic acid, and Glycerol triacetate to mitigate the formation of mHTT aggregates. Following a 48 h incubation period, fluorescence microscopy images displayed a notable decrease in the number and intensity of GFP-tagged mHTT aggregates in the Ponasterone A induced HD150Q cells treated with the fatty acids, in comparison to cells induced with Ponasterone A alone (Fig. 1A). This reduction was visually apparent, indicating a potential modulating influence of the tested fatty acids on mHTT aggregate formation. Subsequently, fluorescence microscopy images encompassing multiple fields were employed to quantify the average number of puncta per field. The results further substantiated the observed decrease in mHTT aggregates. Statistical analysis revealed a significant decline in the number of puncta following treatment with

Decanoic acid, 2-Butyloctanoic acid, and Glycerol triacetate (Fig. 1B).

Western blotting was carried out to validate the findings from fluorescence microscopy. To confirm the reduction of insoluble GFP-tagged mutant huntingtin (mHTT) aggregates following fatty acid treatment, HD150Q cells were incubated with either Ponasterone A (1 μ M) alone or Ponasterone A plus fatty acids at indicated concentrations for 48 h. HTT Protein expression was assessed using an anti-huntingtin antibody. As illustrated in (Fig. 2), insoluble mHTT aggregates increased in cells treated solely with Ponasterone A (see upper panel), while soluble HTT gradually decreased (see lower

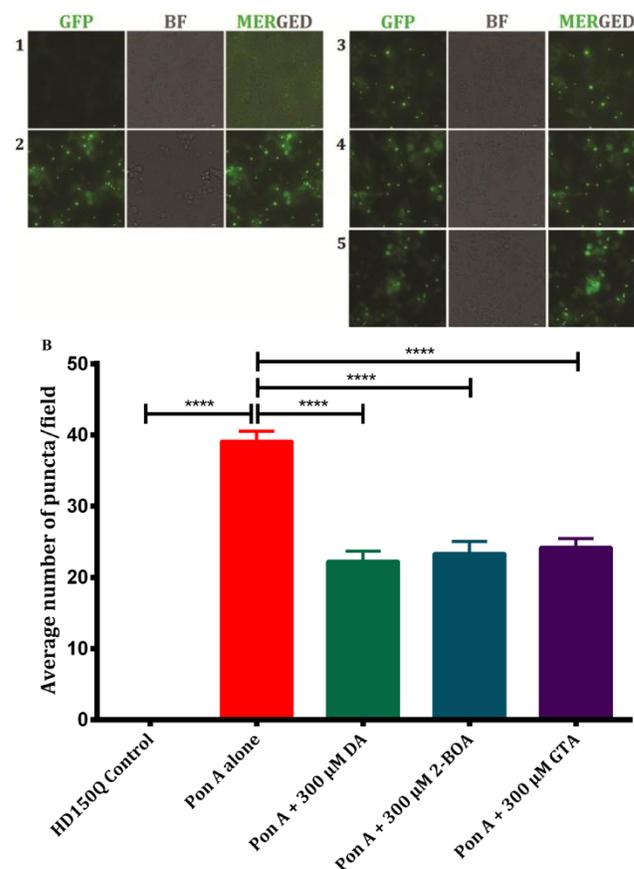


Fig. 1 — (A) 40X fluorescence microscopy images showing GFP tagged mHTT aggregates in HD150Q cells after 48 h of treatments. 1. HD150Q control 2. Ponasterone A alone 3. Ponasterone A + 300 μ M Decanoic acid 4. Ponasterone A + 300 μ M 2-butyl octanoic acid 5. Ponasterone A + 300 μ M Glycerol triacetate. (GFP- Green fluorescent protein; BF- Bright field); and (B): Graphical representation of an average number of puncta per field for microscopy images in figure 1A. Pon A- Ponasterone A; DA- Decanoic acid; 2-BOA- 2-butyl octanoic acid; GTA- Glycerol triacetate); $n=3$; $P \leq 0.0001$ (****)

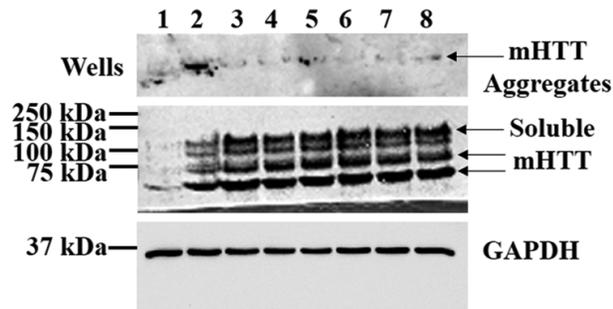


Fig. 2 — Western blot image showing insoluble mHTT aggregates and soluble mHTT in HD150Q cells after indicated treatments for 48 h. 1. HD150Q control 2. Ponasterone A alone 3. Pon A + 100 μ M Decanoic acid 4. Pon A + 300 μ M Decanoic acid 5. Pon A + 100 μ M 2-butyloctanoic acid 6. Pon A + 300 μ M 2-butyloctanoic acid 7. Pon A + 100 μ M Glyceryl triacetate 8. Pon A + 300 μ M Glyceryl triacetate

panel). Interestingly, treatment with various concentrations of fatty acids (100 μ M and 300 μ M) prevented mHTT aggregation and kept the mHTT in soluble form, consistent with the fluorescence microscopy results.

Overall, the results obtained from fluorescence microscopy and western blotting analyses indicate a substantial reduction in GFP-tagged mHTT aggregates in HD150Q cells treated with Decanoic acid, 2-Butyloctanoic acid, and Glyceryl triacetate. These findings suggest the potential of these fatty acids as modulators for preventing and/or reducing mHTT aggregates in Huntington's disease.

Decanoic Acid, 2-Butyloctanoic Acid, and Glyceryl Triacetate abrogates Mitochondrial Reactive Oxygen Species (ROS) levels

To evaluate the effect of Decanoic acid, 2-Butyloctanoic acid, and Glyceryl triacetate on mitochondrial ROS production, HD150Q cells were stained with MitoSOX Red and Mitochondrial ROS levels were visualized and quantified using fluorescence microscope. The stain specifically targets mitochondria and exhibits increased red fluorescence intensity upon oxidation by ROS, enabling reliable measurement of mitochondrial ROS levels. Microscopy images revealed a dramatic reduction in fluorescence signal in the Ponasterone A induced and fatty acid-treated HD150Q cells compared to the cell induced with Ponasterone A alone (Fig. 3A). Quantitative analysis confirmed the significant decrease in mitochondrial ROS levels, indicating the potential of Decanoic acid, 2-Butyloctanoic acid, and Glyceryl triacetate to decrease mitochondrial ROS production (Fig. 3B).

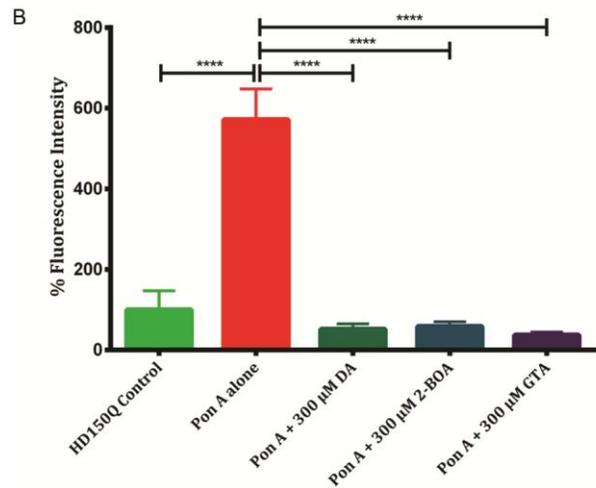
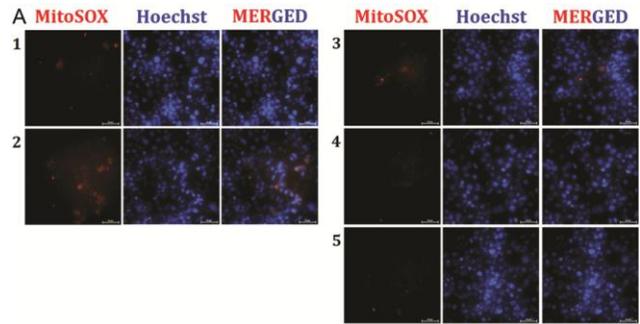


Fig. 3 — (A) 40X fluorescence microscopy images showing Mitochondrial ROS in HD150Q cells after indicated treatments for 48 h. 1. HD150Q control 2. Ponasterone A alone 3. Ponasterone A + 300 μ M Decanoic acid 4. Ponasterone A + 300 μ M 2-butyloctanoic acid 5. Ponasterone A + 300 μ M Glyceryl triacetate; and (B): Graphical representation showing % fluorescence intensity of MitoSOX Red in HD150Q cells after treatment of Ponasterone A with/without fatty acids for 48 h. Pon A- Ponasterone A; DA- Decanoic acid; 2-BOA- 2-butyloctanoic acid; GTA- Glyceryl triacetate) n=3; $P \leq 0.0001$ (****)

Decanoic Acid, 2-Butyloctanoic Acid, and Glyceryl Triacetate Restore ATP Levels

To assess the impact of Decanoic acid, 2-Butyloctanoic acid, and Glyceryl triacetate on cellular ATP production, a luminescent-based ATP determination kit (Thermo Fisher Scientific) was employed. HD150Q cells were treated with Ponasterone A in the presence or absence of varying concentrations of the indicated fatty acids. ATP levels were determined and normalized with protein. As expected, there was a significant reduction in ATP levels upon mHTT expression induced by Ponasterone A in HD150Q cells compared to the un-induced cells, consistent with previous findings of ATP depletion in HD. However, co-treatment with different concentrations of the fatty acids led to restoration of ATP levels in the HD150Q cells (Fig. 4A-C).

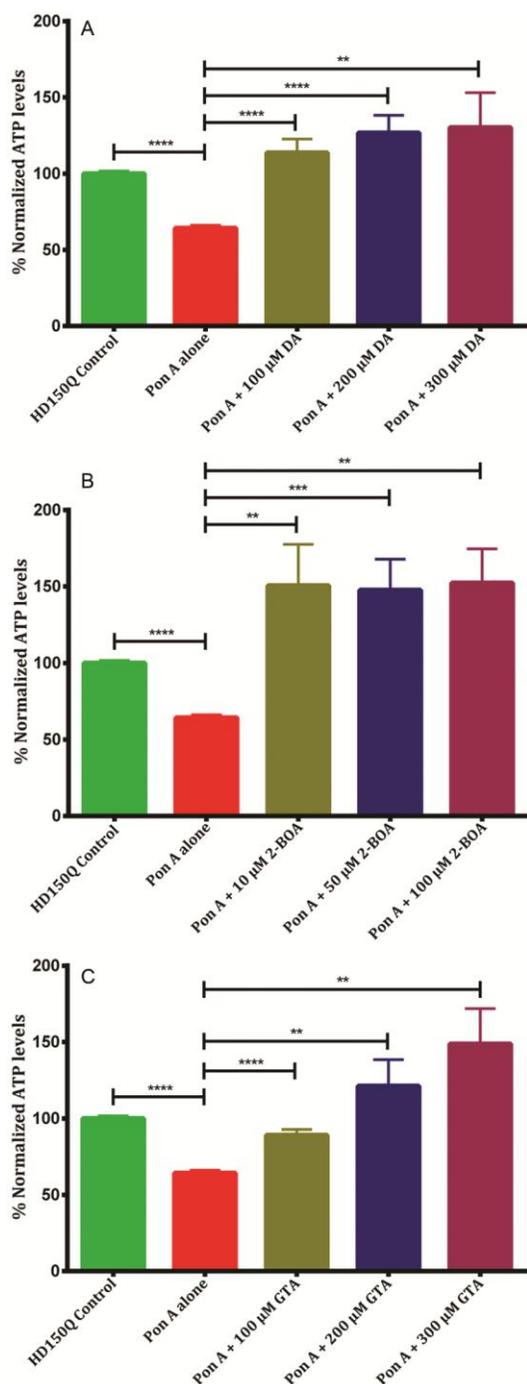


Fig. 4 — (A) Graphical representation of normalized ATP concentrations in HD150Q cells after indicated treatments for 48 h. Pon A- Ponasterone A; DA- Decanoic acid; $n=3$; $P \leq 0.01$ (**); $P \leq 0.0001$ (****); (B) Graphical representation of normalized ATP concentrations in HD150Q cells after indicated treatments for 48 h. Pon A- Ponasterone A; 2-BOA- 2-butyl octanoic acid; ($n=3$; $P \leq 0.01$ (**); $P \leq 0.001$ (***); $P \leq 0.0001$ (****); and (C) Graphical representation of normalized ATP concentrations in HD150Q cells after indicated treatments for 48 h. Pon A- Ponasterone A; GTA- Glyceryl triacetate; $n=3$; $P \leq 0.01$ (**); $P \leq 0.0001$ (****)

Discussion

Huntington's disease is a neurodegenerative disorder that typically manifests in late adulthood and is marked by a range of symptoms, including motor impairments, cognitive deficits, and psychiatric disturbances. Additional clinical manifestations include unintentional weight loss, disruptions in sleep patterns and circadian rhythms, as well as dysfunction of the autonomic nervous system. The absence of a definitive cure for Huntington's disease is widely acknowledged; however, numerous therapeutic interventions exist to alleviate symptoms and enhance the quality of life for affected individuals⁷. Over the past two decades, a total of 99 clinical trials involving 41 distinct compounds have been conducted on patients with Huntington's disease. Regrettably, the progression rate from one stage to the next remains low, with only 3.5% of trials advancing to subsequent phases²⁴.

However, various molecules such as antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) have reached different stages of human clinical trials, as reported by Travessa *et al.* (2017)²⁴. Symptomatic treatment of HD involves the use of different medications, including tetrabenazine (TBZ) and deuterated TBZ (dTbZ), as highlighted by Dash and Mestre (2020)^{25,26}. Nonetheless, the utilization of novel synthetic drugs is accompanied by significant side effects and high toxicity. An intriguing alternative approach involves the use of phytochemicals²⁷ and/or dietary supplements instead of target-specific synthetic drugs for the treatment of neurodegenerative diseases, offering numerous advantages such as minimal side effects and low toxicity to normal cells. The exploration of natural compounds that target multiple cellular processes holds promise for the development of innovative treatment strategies for polyglutamine (polyQ) diseases.

In recent years, fatty acids have emerged as promising neuroprotective compounds and have been explored as therapeutic agents for the treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease²⁸. Specifically, medium-chain fatty acids, including Decanoic acid, as studied by Damiano *et al.* (2020)²⁹, and branched isomers of octanoic acid, such as 2-butyl octanoic acid and 2-butylpropanoic acid, have demonstrated potential in reducing drug-resistant epilepsy²⁸. These fatty acids have also been found to exhibit HDAC

inhibition activity without teratogenic effects, as observed by Eikel *et al.* (2006)³⁰. Moreover, essential fatty acids and sulphur-containing fatty acids, such as Lipoic acid, have demonstrated neuroprotective effects against motor deficits and have been shown to significantly increase survival rates in transgenic mouse models of Huntington's disease³¹⁻³³. However, the precise mechanism by which these fatty acids exert their neuroprotective effects remains unclear. To investigate the neuroprotective effects of two specific medium-chain fatty acids, namely Decanoic acid, 2-butyloctanoic acid and Glycerol triacetate, we employed an inducible cell line model expressing exon 1 of HTT with 150Q repeats.

The findings of this study provide compelling evidence supporting the neuroprotective properties of indicated molecules in an HD150Q cell line model representative of Huntington's disease. The characteristic build-up of mHTT aggregates, oxidative stress, and perturbed adenosine triphosphate (ATP) levels are critical pathological hallmarks associated with HD. Consequently, it is imperative to focus on these aberrant processes in order to devise effective therapeutic strategies for intervention in HD.

The experimental findings unequivocally established that the administration of Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate resulted in a significant decline in the formation of mHTT aggregates within HD150Q cells. These results align with previous investigation that has also reported the capacity to influence protein aggregation dynamics in the context of neurodegenerative disorders. Dirleise Colleet *et al.* (2012) demonstrated that interventions targeting mitochondrial dysfunction, a prominent characteristic of Huntington's disease, such as MK-801, exhibited a correlation with the observed reduction in mHTT aggregates observed in this current investigation³⁴. The observed decrease in mHTT aggregates induced by these fatty acids suggests their potential to impede the aggregation process itself or enhance the clearance of mHTT species from affected cells.

Moreover, the investigation revealed that Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate effectively mitigated oxidative stress by diminishing the production of reactive oxygen species (ROS) within the mitochondria. Oxidative stress, a pivotal factor in the pathogenesis of HD, substantially contributes to neuronal impairment and dysfunction³⁵. Previous studies have reported the antioxidant attributes of specific fatty acids and their derivatives,

corroborating the present findings^{36,37}. For instance, Liberato *et al.* (2012) highlighted the selective peroxisome proliferator-activated receptor (PPAR) γ activation and antioxidant properties exhibited by medium-chain triglycerides, including 2-butyloctanoic acid³⁶. Similarly, Mett *et al.* (2021) demonstrated the neuroprotective effects of Decanoic acid in primary cortical neurons, reinforcing the observed amelioration of oxidative stress observed in the current study³⁷. The ability of Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate to mitigate mitochondrial ROS production underpins their potential as antioxidants or modulators of mitochondrial function. These findings align with earlier investigations that have elucidated the antioxidant characteristics of specific fatty acids and their derivatives^{36,37}.

Another notable finding of this investigation is restoration of ATP levels in HD150Q cells by the administration of fatty acids. ATP depletion is a hallmark of HD³⁸ and contributes to the observed energy deficiency in affected neurons³⁹. The restoration of ATP levels observed with Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate suggests their potential to enhance mitochondrial function and stimulate ATP synthesis. This observation aligns with the study conducted by Chang *et al.* (2013), wherein medium-chain fatty acids associated with a ketogenic diet were shown to enhance seizure control through modulation of ATP production and energy metabolism²⁸. Furthermore, Chang *et al.* (2016) demonstrated that Decanoic acid can safeguard cortical neurons by improving energy metabolism and augmenting ATP production²⁰.

In summary, we provide a compelling evidence for neuroprotective potential of Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate in the HD150Q cell line model of Huntington's disease. The observed ability of these fatty acids to alleviate mHTT aggregates, oxidative stress, and ATP depletion establishes a foundation for subsequent exploration and the formulation of targeted therapeutic approaches for HD. However, it is important to acknowledge that this study relied on a cell line model, necessitating further research to corroborate these results in *in vivo* models and eventually in human clinical trials.

Comprehending the fundamental molecular mechanisms through which these fatty acids exert their neuroprotective effects is of paramount importance to facilitate their translation into clinical

applications. Subsequent investigations should prioritize the elucidation of the precise pathways and targets engaged by Decanoic acid, 2-butyloctanoic acid, and Glycerol triacetate in the context of Huntington's disease.

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Conflict of interest

All authors declare no conflict of interest.

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Clinical studies

F01 ABSTRACT NOT PUBLISHED

F02 A CLUSTER OF HD IN ITALY WITH SEVERAL SUBJECTS CARRYING CAG EXPANSION HOMOZYGOUSITY IN DIFFERENT GENERATIONS AND GENETIC RISK OVER 50%

¹Ferdinando Squitieri*, ²Maria Giovanna Dema, ²Cristiana Borrelli, ¹Federica Consoli, ¹Alessandro De Luca, ¹Simone Migliore. ¹IRCCS Casa Sollievo della Sofferenza (Rome CSS-Mendel), San Giovanni Rotondo, Italy; ²Lega Italiana Ricerca Huntington e malattie correlate (LIRH) Foundation, Rome, Italy

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Background Huntington's disease (HD) prevalence is variable and depends on genetic background. However, culture, environment and other, yet unknown, biological factors may also play a role in determining its frequency. In Italy we estimate a prevalence of 11/100.000 (Squitieri et al., 2016).

Methods LIRH Foundation started to collect clinical and genealogical data together with DNA samples from patients and family members since 2001. Recently, we got in touch with a family from Calabria, Southern Italy, showing a high number of interrelationships and consequent increased risk of transmission of the mutation among people.

Results The family includes about 25 affected people with ascertained HD and hundreds at risk. However, there were two generations of people with homozygous CAG repeat expansion due to two intermarriages of mutation carrier people. The main kindred showed a homozygous mother married with a heterozygous father and 5 children whose four had an homozygous condition. They showed a different HD stage and were included into ENROLL-HD. These people had several 100% risk children.

Conclusion Nevertheless the large experience and the number of publications on genetic and psychological counseling in HD, the scientific community remains still unprepared on how to communicate such unusual risk of HD transmission. These conditions, moreover, contribute to an unpredictable frequency of the disease in some geographical areas.

F03 THE PREVALENCE OF HUNTINGTON'S DISEASE IN BRAZIL

Aparecida Alencar*, Tatiana Henrique. *Universidade Federal de Alagoas/UFAL, S/N, T. do Martins, Maceió, Alagoas, Brasil*

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Background and aims Huntington's Disease (HD) is an inherited neurodegenerative disorder. Despite affecting people in all parts of the world, some countries still have a large gap regarding the diagnosis and consequently the statistical data. While investigation about HD is often in Europe and Asia's countries, in South America, data are still very scarce. The aim of the present study was to verify data on the prevalence of HD in Brazil.

Methods Data collected through the Associação Brasil Huntington (ABH), that registers families of all states in Brazil,

papers about HD in Brazil and field research in Feira Grande city.

Results The Brazilian population presents 207,7 million inhabitants. The only general data currently available on DH in Brazil are concentrated in the records of the ABH, which, according to quantitative data from 2017, present a total of 2481 registered Brazilian families. However, there are records of three clusters with high incidence of DH cases: in the cities of Feira Grande/AL, Senador Sá/CE and Ervália/MG. From these regions, only two have statistical data in the literature: in Feira Grande, the prevalence is 10/10,000 inhabitants (2016); and in Ervália, is 7.2/10.000 inhabitants. Currently, the prevalence in Feira Grande/AL is 10.4/10,000. The limited number of papers suggests that diagnosis is scarce in Brazil, which population is 207,7 millions of inhabitants.

Conclusion The number of families with HD in Brazil, according available data, and some research found suggests that HD, despite the emergence of more research in this regard, diagnosis and records are still scarce. Studies are necessary to elucidate this matter.

F04 GENOTYPE-PHENOTYPE CORRELATION OF HUNTINGTIN CAG REPEAT EXPANSION AND ASSOCIATED SYMPTOMS IN A LARGE INDIAN FAMILY CARRYING HUNTINGTON'S DISEASE MUTATION

¹Roshni Bhatt*, ¹Hitarthi Vyas, ¹Rajubhai Dabhi, ¹Julie Tahilramani, ²Rakesh Shah, ²Suvorit Bhowmick, ¹Ravi Vijayvargia. ¹Department of Biochemistry, The Maharaja Sayajirao University of Baroda, Vadodara, India; ²Vadodara Institute of Neurological Sciences, Productivity road, Vadodara, India; (*) Correspondence: ravi.vijayvargia-biochem@msubaroda.ac.in

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Background We describe a 48 year-old man with a 20 year long history of behavioral changes, chorea and hallucinations. History and mental state examination were suggestive of Huntington disease (HD). Neurological examination revealed discrete, involuntary movements affecting his arms and trunk. Genotyping detected an expanded allele (43 CAG repeats, other allele 17 CAG). A detailed investigation revealed a three-generation-long family history of chorea and psychosis. There was a family history of HD in a sibship of 12 individuals, 9 older than him. The origin of the mutation was traced an ancestral paternal allele. This is a case study to look at an increased expansion in a family where the paternal allele of 43 CAG repeats expanded in the next generation to an allele with 47 repeats.

Method To determine the CAG expansion, blood samples were obtained after informed consent and CAG repeat length was determined by PCR analysis. Till now samples of 5 family members including this patient are processed. To evaluate the impact of CAG repeat expansion on disease progression and symptoms, the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA) and The Stroop Color and Word Test (SCWT) was carried out in these subjects.

Result All the 5 family members were found to carry the HD mutation with the CAG repeat expansion in the affected allele ranging from 43–47. The comparison between age of onset and CAG repeat length and its correlation with motor, cognitive and behavioral analysis are underway.

Conclusion In the current study, we have identified 12 living members of a large Indian family with symptoms of HD. Five patients were confirmed to carry the HD mutation by genetic

testing and remaining members will also be tested and detailed genotype-phenotype analysis will be carried out.

F05 **PREVALENCE OF NON-MOTOR SYMPTOMS IN HUNTINGTON'S DISEASE. A COMPARATIVE STUDY WITH PARKINSON'S DISEASE**

Tatiana Aldaz*, Pasquale Nigro, Celia Painous, Almudena Sánchez, Esteban Muñoz. *Hospital Clinic of Barcelona, Spain*

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Background/aims To assess the prevalence of non-motor symptoms (NMS) in Huntington's disease (HD) and compare it with a cohort of patients with Parkinson's disease (PD)

Materials and Methods Patients were consecutively recruited from the outpatient clinic. They were assessed through the motor part of the Unified Parkinson's Disease Rating Scale (UPDRS-III), the motor part of the Unified Huntington's Disease Rating Scale (UHDRS-motor) and the Total Functional Capacity (TFC). Non-motor symptoms were evaluated using the PD non-motor symptoms quest (NMSQuest) in both HD and PD patients.

Results We enrolled 123 participants: 53 HD, 45 PD and 25 healthy controls (HC). NMS were significantly more prevalent in HD patients than in HC. The most frequent NMS in HD were attentional deficits (73.58%), apathy (64.15%), dysphagia (62.26%), memory complaints (62.26%), depression (56.60%) and falls, insomnia and urinary urgency (50.94%). There was not statistical difference in the total score of NMS between early (1–2) vs. advanced stages (>3). Only dysphagia and memory complaints were more prevalent in the latter. The total score of the NMS was higher in HD than in PD ($p=0.025$) even though the duration of the disease was similar in both groups. HD scored significantly more than PD in 11 items (dysphagia, constipation, fecal incontinence, fecal tenesmus, weight loss, memory, apathy, attention, falls, nightmares, illusions).

Conclusions Patients with HD have a high prevalence of non-motor symptoms compared with HC. There is little difference between initial and advanced stages. The prevalence of non-motor symptoms is higher in HD than in PD.

F06 **WHEN AND HOW DOES MANIFEST HD BEGIN? A COMPARISON OF AGE AT ONSET OF MOTOR AND NON-MOTOR SYMPTOMS**

^{1,2}Herwig Lange*, ¹Stefan Bohlen, ¹Ralf Reilmann. ¹George Huntington Institut Münster, Münster, Germany; ²Reha-Zentrum Dinslaken im Gesundheitszentrum Lang, Dinslaken, Germany

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Background In his classical paper, George Huntington described movement disorder and psychiatric symptoms. In 1915, Davenport described 'a biotype in which the tremors are absent but mental deterioration present'. In 1921, Entres used the term 'chorea sine chorea' to depict onset of HD without motor symptoms. Until today diagnosis of HD without overt motor symptoms is an unmet need.

Aims Present corroborated data on age at onset and onset of clinical symptoms (motor, cognitive, behavioral) as available form charts of patient seen at the GHI out-patient clinic.

Methods/techniques SPSS analysis of age at onset of symptoms of 260 symptomatic HD patients (DCL=4) available for analysis at GHI before December 2017.

Results/outcome In those 260 patients both age at diagnosis and at onset of motor symptoms was documented. In 10 patients of this group, HD was diagnosed in an external center before documented motor onset. In 9 of these cases, diagnosis was established within 1 year after motor onset. Depression was documented in 196 of the 260 patients. Depression was present in 32% before the onset of motor symptoms and was the symptom that appeared more frequently before motor symptoms than any other symptom. Cognitive problems were documented in 211 cases, but occurred in only 15% before the onset of motor symptoms.

Conclusion New guidelines are needed to enable clinicians to provide adequate care early and establish a reliable diagnosis of HD in the absence of overt motor symptoms, permitting access to health care support.

F07 **A FREQUENCY OF CONCOMITANT DISORDERS IN PRESYMPTOMATIC HUNTINGTON'S DISEASE PATIENTS**

¹Elżbieta Alicja Puch*, ²Grzegorz Witkowski, ³Mark Isalan, ^{3,4}Michał Mielcarek, ⁴Daniel Zielonka. ¹Department of Human Evolutionary Biology, Adam Mickiewicz University, Poznan, Poland; ²First Department of Neurology, Institute of Psychiatry and Neurology, Warszawa, Poland; ³Department of Life Sciences, Imperial College London, Exhibition Road, London, UK; ⁴Department of Epidemiology of Rare Diseases and Neuroepidemiology, Poznan University of Medical Sciences, Poznan, Poland

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There is growing evidence that Huntington's disease (HD) is a multi-system disorder. Although HD is predominantly recognized as a neurodegenerative disease, there is a mounting evidence that peripheral component to its pathology may also contribute to the disease progression. A number of pre-clinical studies in recent years revealed that murine models of HD develop a wide range of peripheral tissue abnormalities including eyes, cardiac and skeletal muscles, even at the pre-symptomatic stage.

Hence, the aim of this study was to unravel concomitant disorders in the pre-symptomatic HD subjects.

Our study was performed based on two EHDN Study sites in Poznan and Warsaw (Poland) and we collected data about: a frequency of concomitant disorders, age and number of CAG repeats from the REGISTRY and ENROLL-HD programs. In total, we identified 97 presymptomatic individuals using UHDRS as a criterion out of 542 patients between those two EHDN centers.

The mean of age for the presymptomatic patients was 32.5 (\pm SD 8.9) years while the mean of the number of CAG repeats was 42.6 (\pm SD 3.1). We found that nearly a third of the group (28.9%) did not report any concomitant disorders. However, there was a number of patients that already developed psychiatric diseases (26.8%), predominantly depression was present in 18 cases. Moreover, we found the following comorbid conditions: musculoskeletal disorders and injuries (19.6%), allergies (18.6%), cardiovascular disorders (14.4%), neurological disorders (13.4%), gastrointestinal disorders (9.3%) and thyroid abnormalities (8.2%). A comparative analysis showed that the same comorbidities were observed in the symptomatic and presymptomatic HD subjects, only with a lower frequency in the latter.