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Long-term administration of omega-3 fatty acids alleviates Angelman syndrome-like phenotype in an *Ube3a* mutant strain of *Drosophila melanogaster*

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ABSTRACT

Angelman syndrome (AS) is a complex and relatively frequent genetic disorder that disturbs the nervous system, with severe outcomes in children and adults. *Drosophila melanogaster* is a well-established experimental model, widely utilized for demonstrating the biological mechanisms involved in various human neurodegenerative diseases. Here, we successfully generated and described a new *Ube3a* mutant strain of *D. melanogaster* exhibiting AS-like phenotypes, such as locomotor disabilities, a condition seriously aggravated in homozygous male mutants. In order to salvage the AS-like phenotype, *Ube3a* mutant and normal strains of *D. melanogaster* were subjected to a long-term dietary supplementation with eicosapentaenoic and docosahexaenoic omega-3 fatty acids found in commercially available capsules containing fish oil. Although essential for the normal development of the nervous system in many animals, omega-3 fatty acids proved strong positive effects on the climbing abilities of the homozygous male mutants. This paper represents the first report regarding the impact of omega-3 fatty acids on the climbing phenotype of an AS-like *D. melanogaster* mutant and brings relevant knowledge to the continuously developing field of nutrigenomics.

Keywords: *omega-3 fatty acids, Ube3a, Angelman syndrome, Drosophila melanogaster, locomotor performances.*

1. INTRODUCTION

Drosophila melanogaster is a very suitable model for studying human neurodegenerative diseases due to the evolutionary conservation of key mechanisms involved in the development, functioning and maintenance of nervous tissues [1-4].

Ube3a gene from *D. melanogaster* is orthologous with *UBE3A* human gene, the latter being the main determinant of Angelman syndrome (AS), a relatively common human neurogenetic disorder, severely affecting the normal development of the central nervous system. The main clinical phenotypic traits of AS include mental retardation, locomotor impairment and frenzied movements [5]. The complete deletion or different types of mutations that affect the function of *UBE3A* are among the main causal factors for AS [6]. Both *UBE3A* and *Ube3a* act as main players in the ubiquitination molecular processes and are responsible for the intracellular degradation of proteins, and the expansion and correct function of neurons since the early stages of embryonic development [7, 8].

Ube3a is involved in various biological processes such as protein catabolism, locomotion, long-term memory, circadian rhythm, morphogenesis of dendrites and development of neurons in the peripheral nervous system [9-11]. The catalytic domain of the ubiquitin protein ligase E3A protein (*Ube3a-PA*) has a 62% identity with the corresponding domain of the homologous human protein [12]. Besides modulating the neuronal homeostasis processes, *Ube3a* influences ATP synthesis and metabolism, and also the production and structural stability of the actin present in the cytoskeleton [13]. Mutant alleles of *Ube3a* lead to various phenotypes that range from mild, such as gravitaxis and behavioral deficiencies, to more severe phenotypes, which strongly affect the mobility of individuals and the development of dendritic branches. Particular alleles can even induce lethality [10].

Omega-3 polyunsaturated fatty acids (PUFAs) are essential for normal development and health maintenance of many animal species. These are involved in maintaining the biophysical properties of the membrane, the activity of membrane channels, and the regulation of gene expression via nuclear receptors [14]. Omega-3 PUFAs added to the diet of several human patients suffering from central nervous system disorders (including patients with AS) led in several cases to the significant decrease of the frequency and intensity of epileptic episodes [15]. So far, however, no additional rigorous and systematic studies have been conducted in order to attest the efficacy of this treatment [16]. *In vitro* experiments performed on rat neurons pre-treated with omega-3 have demonstrated their increased resistance when exposed to stressful conditions, such as lack of oxygen and glucose depletion. Transgenic mice that manage to transform omega-6 to omega-3, as well as the mice whose diet were supplemented with omega-3, are more resilient to cerebral ischemia compared to control mice [17]. Diet supplementation with omega-3 had positive effects on cognitive function also in *Rhesus macaque* [18].

The aim of this study was the generation and phenotypic characterization of *D. melanogaster* individuals harboring a new *Ube3a* mutant allele (symbolized $As^{m1.5\text{-}R}$) and the subsequent investigation of the influence of omega-3 PUFAs on the neurological function of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males. In order to achieve

the latter objective, we set up a long-term experimental feeding procedure that supposed the supplementation of the *Drosophila* regular culture medium with eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) long-chain PUFAs, which are found in commercially available capsules containing fish oil. Our results suggest that $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$mutant males exhibiting severe locomotor deficiencies gain significant mobility comparable to that of wild-type *D. melanogaster* malesconsecutive to omega-3 continuous feeding.

2. EXPERIMENTAL SECTION

2.1. *Drosophila* strains.

In the present study, we used *D. melanogaster* males from *Oregon* wild-type (or reference) strain and from $As^{m1.5\text{-}R}$ mutant line. For the generation of $As^{m1.5\text{-}R}$ line [19], the $P\{EP\}Ube3a^{EP3214}$ transposable element residing within the 5'UTR of *Ube3a* gene was mobilized in individuals pertaining to *EP(3)3214* transgenic line [20] using a *Δ2-3* transposase source [21].

The cultures of *D. melanogaster* were kept at 18-20ºC, under a natural-like light/darkcycle on regular corn-yeast-agar medium or, following the experimental setup, on regular medium supplemented with either 500 µl of absolute ethanol or with a mixture of omega-3 PUFAs enriched fish oil mixed with 500 µl of absolute ethanol.

Selection and scoring of the flies were achieved using both an Olympus SZ61 and Olympus SZX7 stereo microscopes.

2.2. Experimental feeding with omega-3 rich fish oil.

Commercially available alimentary supplements containing omega-3 fatty acids were purchased from *Walmark*, Trinec, Czech Republic (http://www.walmark.eu/). The package contains capsules, each enclosing 1000 mg of fish oil obtained from selected individuals raised in strict ecological conditions. The 1000 mg of fish oil contains 30% EPA and 20% DHA. To 100 ml of *Drosophila* regular medium was added either 500 µl of absolute ethanol (standing for the standard feeding conditions) or an *ad-hoc* prepared mixture comprising of 1000 mg of fish oil (having 300 mg of EPA and 200 mg of DHA, respectively) and 500 µl of absolute ethanol (the omega-3 experimental feeding conditions) [22]. The medium was distributed in 240 ml culture polypropylene bottles (Flystuff, Genesee Scientific Corporation) or comparable glass recipients, each containing roughly as 20 ml of medium. The bottles were closed with a thick cotton wool plug and kept until usage at 4ºC avoiding light exposure, in order to minimize the oxidative rancidity.

Three distinct cultures for each experimental setup (standard or omega-3 supplemented medium) were started using (20 females + 10 males)/culture gathered from *Oregon* and, respectively, $As^{m1.5\text{-}R}$ lines. The *Drosophila* cultures were transferred in recipients containing fresh medium on a monthly basis. After approximately six months, due to the very sensitive experimental conditions, certain interchange of flies between the cultures replicas of individual experimental setups was required. Nevertheless, during the entire period of experimental feeding (more than a year), we did not add external *Drosophila* individuals in any of the four types of culture.

2.3. Molecular biology methods.

Genomic DNA was extracted using an adapted protocol [23]. PCR amplifications of the genomic region encompassing the $P\{EP\}Ube3a^{EP3214}$ original insertion site were performed using the primers symbolized A3 (5'agtgcaaacatccaacggac3') and A5 (5'aacgcttcatttcgggcgtg3'). The primers allow obtaining a 628 bp amplicon when using a wild-type DNA template and an 882 bp amplicon when using a DNA template isolated from homozygous $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ individuals. The amplicons were purified from a 2% agarose gel using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing was carried out with both A3 and A5 primers in a Beckman CEQ8800 automated DNA sequencer using the GenomeLab DTCS Quick Start Kit (Beckman Coulter). A partial nucleotide sequence of the regulatory 5'UTR region of $As^{m1.5\text{-}R}$ allele was indexed in NCBI (GenBank accession number GU120633).

2.4. Bioinformatics.

The nucleotide sequences were aligned against the *Drosophila* genome (release R5.57) using both Genome ARTIST [24] and BLAT from UCSC [25]. The sequence features were identified and annotated by using CLC Main Workbench 6.9 software (CLC Bio-Qiagen, Aarhus, Denmark).

2.5. Climbing assay.

The climbing assay aimed to test the effects of omega-3 long term diet supplementation on the *Drosophila* AS model represented by flies harboring the $As^{m1.5\text{-}R}$ mutant allele of *Ube3a* gene.

In order to achieve this, we sought to identify if there are significant differences between the climbing abilities of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males (fed with standard or omega-3 supplemented medium) and *Oregon* males (feed with standard medium). We performed two locomotor performance analyses, each executed during the beginning of springtime, and therefore seasonal comportment biases were at least partially circumvented.

During the **first climbing** experiment, 15 Oregon and 10 $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ adult males (about 18-20 days old) were collected from cultures with regular medium and further kept in separate glass small bottles filled with 5 ml of medium until employed in experiments. The mobility testing was performed at noon; each group of males was introduced in 16 mm/160 mm transparent glass tubes that were closed with cotton wool plugs, and then vertically introduced in small indents made in a thick piece of polystyrene. The test tubes were marked at 5 and respectively 10 cm distance from the bottom. The piece of polystyrene was gently tapped on the table; when all the flies from the testing tubes were at the bottom, they were allowed to climb for about 15-16 seconds, and then the tapping procedure was repeated. There were performed two sets of 20 climbs, separated by a 30 minutes break.

The **second climbing** assay was performed at four years distance from the first one, using similar experimental settings.

The flies were collected from two distinct cultures of each of the selected experimental setups (standard or omega-3 supplemented medium - see 2.2), after more than a year of controlled feeding. Eight *Oregon* males raised on standard feeding medium were tested against two distinct groups of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males comprising of 8 mutant males raised on standard medium and 7 males raised on omega-3 supplemented standard medium, respectively. Consecutive to their collection, the *Drosophila* males were kept on regular medium until they were used in the locomotor tests (at the age between 18 to 20 days). The climbing procedure consisted in three sets of 10 climbs, separated by five minutes pauses.

The mobility assessment experiments were performed using natural light and the flies were filmed using an Olympus SP-350 digital camera. The recorded climbing data were analyzed without knowing the identity of the flies from a given test tube. We quantified the number of males from within each group that climb above the 5 cm mark in a 10 seconds interval.

2.6. Statistical analysis.

The number of flies that performed a successful climbing was divided by the total number of flies comprising their particular group. The resulted percent values were clustered in corresponding data sets that were compared by means of the unpaired t test with Welch's correction using GraphPad Prism version 5.04 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).Within the article,data are expressed as mean ± 95% CI.

3. RESULTS AND DISCUSSION SECTION

3.1. Phenotypic characterization of $As^{m1.5\text{-}R}$ mutant line.

Phenotypic analysis of the $As^{m1.5\text{-}R}$ line revealed that the $As^{m1.5\text{-}R}$ mutant allele is viable when harbored by females (in one or two copies), but determines a high level of mortality in the case of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ homozygous males. Actually, a comprehensive evaluation performed on 4388 individuals from $As^{m1.5\text{-}R}$ line identified only nine $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males, thus indicating an escaper rate of approximately 2 percent. The other expected genotypic categories followed a Mendelian distribution. Previous studies revealed that experimental over-expression of *Ube3a* using the pan-neuronal driver *elav-Gal4* is lethal only for *Drosophila* males, a case presenting strong similarities with the male-biased severe phenotypic consequences determined by $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ genotype [10]. Additionally, we observed that few mutant homozygous females have an extra bristle on one humerus, a phenotype resembling the *Humeral* dominant mutation affecting *Antennapedia* gene [26]. Prior to performing focused studies on this regard, we also noticed by casual observation that homozygous individuals exhibit a certain degree of locomotion difficulties.

3.2. Nucleotide sequence features of $As^{m1.5\text{-}R}$ allele.

Sequencing of the A3 + A5 amplicon obtained using $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ genomic DNA (see 2.3, Experimental section) revealed that $As^{m1.5\text{-}R}$ allele is characterized by the presence of a 246 bp *P{EP}* remnant within the 5'UTR region of *Ube3a* gene (Figure 1). The *P{EP}* fragment, hereby symbolized *P{EP}-As*, is in opposite orientation relative to the sense strand of *Ube3a* and starts with 227 nucleotides pertaining to the canonic *P{EP}*3' end and continues with an additional 19 nucleotides from the 5' end of *P{EP}*. *P{EP}-As* is bordered by two identical genomic nucleotide octets (ACTTACGC), the supplementary octet resulting consecutive to a transposition site duplication (TSD) event that accompanies the insertion of P-element [27]. The coordinates of the remnant localization within the reference sequence of *Ube3a* should be considered both 11203597 and 11203604, according to the mapping paradigm argued elsewhere [24, 28].

The over-expression of *Ube3a* was found to induce lethality in males [10]. The nucleotide sequence of the *P{EP}* remnant was scanned with MOTIF Search on-line application (http://motif.genome.jp/) aiming to identify within it potential transcription factors binding sites (TFBSs) (data not shown); frequently, their presence indicates enhancer trap properties. This approach revealed that *Deformed* (*Dfd*) transcription factor has several TFBSs within *P{EP}-As* sequence. *Dfd* is a homeotic gene, component of the *Antennapedia* complex (*Ant-C*), which is active since the early stages of embryo development [29] and it is vital for the neuronal differentiation in the developing brain of *D. melanogaster* [30]. For a more detailed analysis regarding the TFBSs of *Dfd* in the 5'UTR regulatory region of the $As^{m1.5\text{-}R}$ allele, we used CLC Main Workbench 6.9 software (CLC Bio-Qiagen, Aarhus, Denmark). In order to achieve our goal, we ran the "Find motif" task using the canonical *Dfd* binding sequence motif *NNNNNNATTAMYNNNN* [31]. Four novel TFBSs (three having the plus orientation and the forth having the minus orientation in regard to the sense strand of *Ube3a*) were identified within *P{EP}-As*, a particularity that rises to five the number of *Dfd* motifs within the 5'UTR of $As^{m1.5\text{-}R}$ allele (Figure 1). These TFBSs are distributed within a 100 nucleotides window, defining a theoretically functional hot-spot for *Dfd* coupling. If such a scenario is true, it could lead to $As^{m1.5\text{-}R}$ allele activation, as proved by past studies that found an increase of *Dfd* binding when characteristic TFBSs were artificially introduced in referential genomic environments [32]. This process was followed by specific reporter gene activation.

3.3. Climbing assays results.

The $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ genotype determines a male-biased lethality, thus only few mutant homozygous escaper males live and can be subjected to behavioral tests. During the initial climbing assay, each set of trials consisted in 20 vertical races. Climbing data analysis revealed that there is a significant decrease of mobility in the control *Oregon* group when the first ten runs are compared with the last ten runs ($p = 0.024$), a tendency also confirmed on *Oregon* females (data not shown). In the light of these observations, we decided to use for the statistical analyses only the results of the first ten climbs executed in each set of trials. The statistical analysis showed a very significant mobility

decrease in $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males compared to the *Oregon* control males ($p < 0.0001$), indicating a possible neuronal impairment in *Ube3a* mutants (Figure 2A). The differences between control and mutant males become even more evident if a methodical analysis of the climbing sessions is performed. For example, during the fifth climb from the first set of locomotor tests, after five seconds almost half of the total number of *Oregon* control males completed the vertical race, a task accomplished after 10 seconds by more than 90 percent of controls (Figure 3, test tube number 1). In contrast, the $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males are slower, none of them completing the trial within the first 5 seconds and only 50 percent finalizing it after 10 seconds (Figure 3, test tube number 2). Some standard climbing assays number the individuals that reach the top of the testing tube after roughly 18 seconds [33]. Considering this strategy, we also scored the locomotor performances from the fifth race after 15 seconds of free moving. Not even one $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ male reaches the top of the test tubes in the 15 seconds interval, a strikingly weaker performance compared with the *Oregon* males (Figure 3).

In the second experimental set up, selected continuous lineages of $As^{m1.5\text{-}R}$ mutants were systematically fed for slightly more than a year with commercially available fish oil rich in EPA and DHA omega-3 fatty acids.

Experimental feeding with omega-3 fatty acids has documented effects on different biological processes, including cancer prevention [34]. In order to express the beneficial influences, omega-3 PUFAs need to be protected by oxidative processes leading to rancidity [35]. Studies performed on fish oil stability without antioxidants additions have demonstrated that the fish oil still has normal peroxide and anisidine values for about 36 days when stored at relatively low temperatures and consecutive to daily short exposure to oxygen [36]. If more strict conditions are used, it can be used for over 60 days [35]. We maintained our omega-3 supplemented *D. melanogaster* cultures in a chill environment and with reduced oxygen exposure and changed the culture medium on a monthly basis (see the Experimental Section). In these conditions, we believe that the rancidity effects were kept at adequate values and we scored the real effects of omega-3 fatty acids consumption.

During the spring of 2015, new climbing assessments were performed using *Oregon* males fed with standard experimental medium and two groups of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males collected from standard medium and omega-3 supplemented medium, respectively. The results of the comparisons between the three male groups are intriguing (Figure 2B). As expected, $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males raised on standard medium exhibit significantly impaired mobility compared to *Oregon* males ($p = 0.0004$). Meeting our expectations, the climbing performances increased within the $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ male group raised on medium containing EPA and DHA omega-3 fatty acids. More precisely, they experimented a mobility similar with that of *Oregon* control males and significantly higher than that of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males raised on standard medium ($p = 0.0035$). Although confirmation of this result is necessary, to the best of our knowledge, this is the first direct proof of a beneficial effect of EPA and DHA omega-3 fatty acids consumption on a mutant *D. melanogaster* phenotype.

The $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males exhibit severe phenotypes, such as significant locomotion impairment or lethality, resembling previously documented effects of *Ube3a* malfunctioning [10, 11].

In mice, *Ube3a* targets the synaptic protein *Arc*, which regularly interacts with *endophilin* in order to promote synaptic plasticity [37]. On the other hand, studies in *Caenorhabditis elegans* and *D. melanogaster* showed that *endophilin* is required for the recruitment of synaptojanin (encoded by *Synj* gene) to sites of neurotransmitter release [38, 39]. Altering the regular function of *Synj* in experimental mice leads to vesicle cycling defects that are very similar to those observed in a mouse AS model [40]. Corroborating these data, we propose that in *D. melanogaster*, *Ube3a* gene could strongly influence particular downstream effectors, such as *Arc*, *endophilin* and *Synj*, thus becoming plausible that developing certain neurological defects could be attributed to *Ube3a* distorted functioning.

The effects of introducing unsaturated fatty acids into the alimentation of different animal species are very complex. Studies performed on different mutant *C. elegans* individuals fed with omega-3 PUFAs demonstrated positive effects for efficient neurotransmission [41], the regulation of synaptic vesicle recycling [42] and normal alcohol response behaviors [43]. Omega-3 diet supplementation led to the upregulation of the adult neurogenesis in healthy specimens oflobster (*Homarus americanus*) [44]. Additional studies performed on mice emphasized the important role played by omega-3 in adult hippocampal neurogenesis [45].

D. melanogaster does not naturally need or synthesize EPA and DHA, the two omega-3 PUFAs not being found by targeted biochemical analyses [46]. However, the same study demonstrated that individuals fed with omega-3 fatty acids supplements successfully incorporate them. Remarkably, the adults converted about 85% of DHA to EPA, the males being more efficient than the females. Omega-3 PUFAs are not eliminated consecutive to experimental feeding procedures [46], thus allowing us to hypothesize that after being ingested by *D. melanogaster* individuals they could exert similar functions as those demonstrated within other animals.

The majority of dietary studies involving *D. melanogaster* highlighted especially the effects of C18 fatty acids on models of neurodegenerative diseases and hypercholesterolemia [47], on alcohol toleration [48], on fitness traits and oviposition preferences [49], and on mitochondrial fusion and function [50]. Our original experimental design was aimed to lighten the locomotor impairments of *D. melanogaster* adults exhibiting AS-like phenotypes, such as slow basal motion and abnormal climbing abilities, by EPA and DHA dietary supplementation. Similarly, human adults that are affected by AS undergo epileptic seizures and severe locomotor complications, while many of them need constant help for accomplishing regular tasks and being wheelchair-bound [51].

The $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males treated with omega-3 PUFAs apparently have a normal behavior. Previous studies acknowledged that EPA is the main factor which positively influences the behavior and mood in humans [52], while in *C. elegan s*it rescues the slow basal speed phenotype of adult *fat1* and *fat3* mutants, consecutive to continuous supplementation of the

food throughout every developmental stage [43]. Considering the aforementioned studies and since Shen *et al*. [46] demonstrated in *D. melanogaster* that about 85% of DHA is converted in EPA, it is reasonable to assume that the behavioral improvements of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males may be allotted mainly to EPA consumption effects. In addition to other mechanisms of action, EPA could stimulate the localization of synaptojanin at neurotransmitter release sites, as previously demonstrated in *C. elegans* [42]. As the mutant *Ube3a* genomic background may affect *Synj* function, omega-3 fatty acids (mainly EPA in *D. melanogaster*) dietary supplementation can partially alleviate this effect and rescues the locomotor impairments of the $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ mutant males.

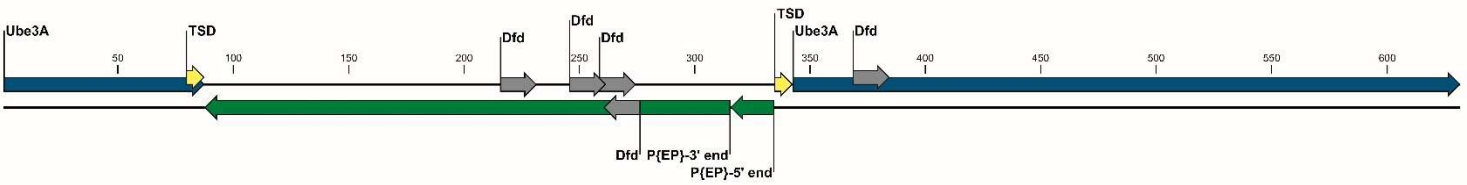

Figure 1. The schematic representation of the 5'UTR (blue arrows) of *Ube3a* gene containing the *P{EP}-As* remnant (green arrows), which defines $As^{m1.5\text{-}R}$ allele. With yellow arrows are indicated the two TSDs, while the grey arrows stand for the TFBSs of *Dfd*. The *P{EP}* fragment is in opposite orientation relative to the *Ube3a* gene orientation.

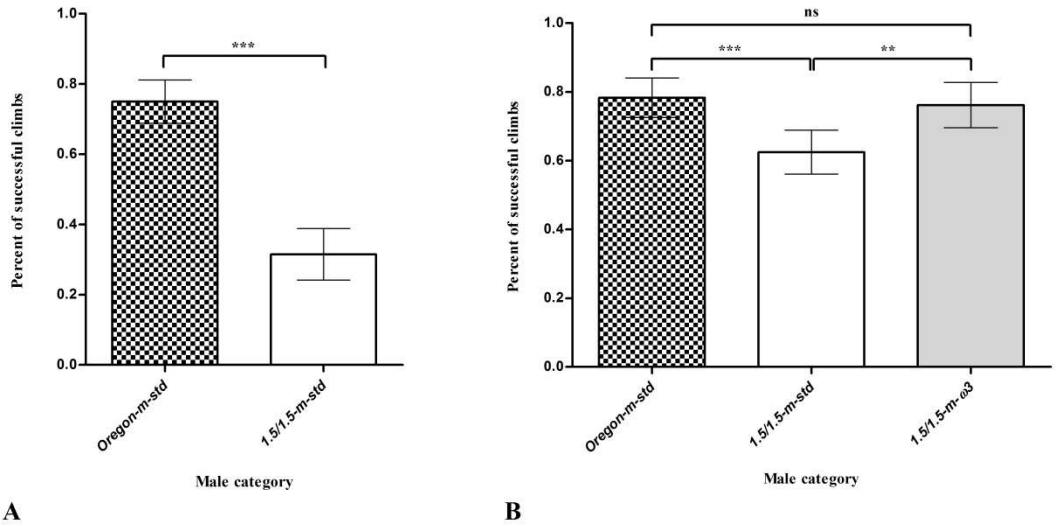

Figure 2. The climbing assays performed using *Oregon* control males raised on standard medium (*Oregon-m-std*) versus $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ mutant males raised on standard (*1.5/1.5-m-std*) or omega-3 supplemented medium (*1.5/1.5-m-ω3*). *Oregon-m-std* performed better than *1.5/1.5-m-std* in both climbing assays, while the one-year supplementation of the feeding medium with omega-3 fatty acids (Figure 2B) resulted in a significant increase of *1.5/1.5-m-ω3* climbing performances to a level comparative to that of the control. The error bars stand for the 95% confidence interval. **, $p < 0.005$; ***, $p < 0.0005$; ns, not significant.

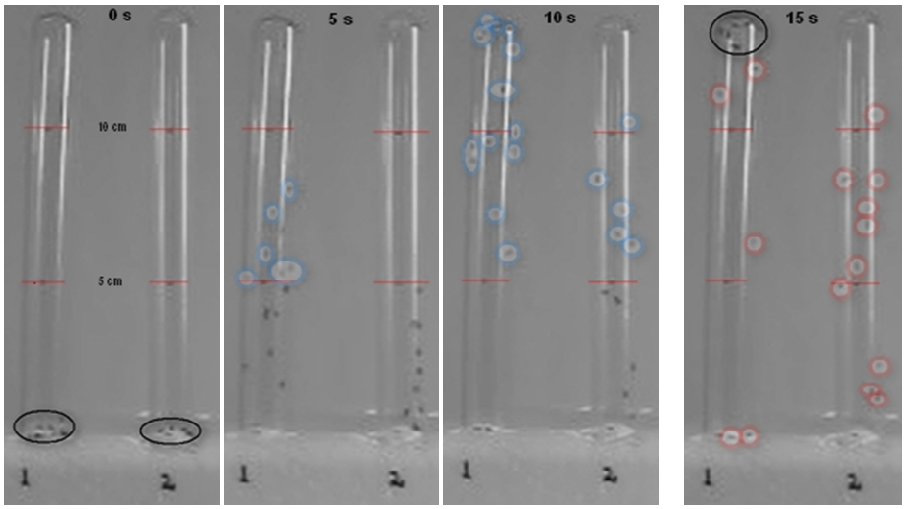

Figure 3. The image illustrates in chronological frames the climbing behavior of *Oregon* control males (tube 1) versus $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ mutant males (tube 2). At time 0 (0 s), both males groups are at the bottom of the test tubes; five seconds later (5 s), 40% of the *Oregon* males already completed the 5 cm run (indicated by blue rings), while none mutant males crossed the 5 cm mark. At the end of the trial (10 s), 93% of the *Oregon* males accomplished the climbing challenge, while only half of the mutants successfully climbed above 5 cm. An additional 5 seconds interval (15 s) reveals that only about 25% of the *Oregon* males are not at the top of the test tube (indicated by red rings), while the mutant males are scattered along the test tube, none reaching the top.

4. CONCLUSIONS

In the current study we successfully generated an AS-like *D. melanogaster* model and tested the effects of omega-3 fatty acids dietary supplementation on the performances of individuals fitting to this model. By means of *P{EP}* transposon mutagenesis, we generated an *Ube3a* mutant strain, symbolized $As^{m1.5\text{-}R}$, which exhibits several phenotypic traits reminding of the typical AS human patients having mutations in the orthologous *UBE3A* gene, such as slow motion and abnormal locomotor behavior. To our surprise, these mutant phenotypes were more severely manifested in $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males, which seldom reach adulthood, the majority dying within pre-pupa developmental stages. PCR amplifications and subsequent sequencing of the target amplicons allowed us to find that the specific $As^{m1.5\text{-}R}$ allele has a *P{EP}-As* remnant inside the 5'UTR of *Ube3a* gene. Bioinformatic analysis of the *P{EP}-As* residue indicated that it contains a putative hot-spot for *Dfd* transcription factor binding, which could seriously affect the expression of *Ube3a*, hence leading to the characteristic mutant phenotypes.

Long-term dietary supplementation with EPA and DHA omega-3 fatty acids enriched commercially available fish oil succeeded to rescue the locomotor impairments of $As^{m1.5\text{-}R}/As^{m1.5\text{-}R}$ males, as revealed by carefully designed climbing assays. A meticulously literature assessment endorsed us to propose that the beneficial effects of omega-3 fatty acids consumption could be primarily due to EPA, which might promote the synaptojanin role even if it is disturbed in an *Ube3a* mutant genomic background.

To our best knowledge, the studies detailed here are the first demonstrating a direct beneficial effect of omega-3 fatty acids consumption in a *D. melanogaster* neurologic mutant. Although further inquiries addressing this topic are needed, our results could inspire a reconsideration of the potential that EPA and DHA omega-3 fatty acids dietary consumption could have for remedying neurodegenerative human disorders. Furthermore, a more careful analysis of the possible EPA and DHA specific molecular targets might be critical for understanding particular aspects of the fast developing field of nutrigenomics.

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