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Short communication

Long-term efficacy of docosahexaenoic acid (DHA) for Spinocerebellar Ataxia 38 (SCA38) treatment: An open label extension study

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ABSTRACT

Introduction: Spinocerebellar Ataxia 38 (SCA38) is caused by *ELOVL5* gene mutation, with significant reduction of serum docosahexaenoic acid (DHA) levels. DHA supplementation has been proven effective at short-term follow-up. In the present paper, we evaluated long-term safety and efficacy of 600 mg/day oral DHA in SCA38 by a 2-year open label extension study.

Methods: Nine SCA38 patients underwent standardised clinical assessment at 62 (T1), 82 (T2) and 104 (T3) weeks, and compared to pre-treatment scores (T0). Brain 18-Fluorodeoxyglucose Positron Emission Tomography and electroneurography were performed at T0 and T3.

Results: We found a significant maintenance of clinical symptom improvement at each follow-up time-point ($p < 0.001$) as compared to T0, a sustained increase of cerebellar metabolism at T3 as compared to T0 ($p = 0.013$), and no worsening of neurophysiological parameters. No side effect was recorded.

Conclusions: Long-term DHA supplementation is an eligible treatment for SCA38.

1. Introduction

We have recently suggested supplementation with docosahexaenoic acid (DHA) as an effective treatment in Spinocerebellar ataxias 38 (SCA38), an autosomal dominant disorders phenotypically characterized by gait and limb ataxia, followed by dysarthria, dysphagia, and ophtalmoparesis, and in which *pes cavus* and hyposmia may be considered distinctive associated features [1,2].

SCA38 is caused by mutations in the *ELOVL5* gene [3], which encodes an elongase enzyme involved in the synthesis of very long-chain fatty acids with a high and specific expression in Purkinje cells [4]. The mutations lead to a reduction of serum DHA of the omega-3 polyunsaturated fatty acid class [3].

Given the reduction of DHA levels in SCA38 patients, we have previously evaluated the safety and efficacy of DHA supplementation, and we carried out a double-blind randomised placebo-controlled study for 16 weeks, followed by an open-label study with overall 40-week DHA treatment. We demonstrated that oral DHA supplementation is a safe and effective treatment, exerting clinical efficacy and ameliorating cerebellar metabolism [2].

Here, we report the results of the subsequent long-term open-label extension study, aimed at evaluating the long-term safety and efficacy

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of oral DHA supplementation (600 mg/day) on clinical disease course, cerebellar metabolism and neurophysiological parameters up to two years follow-up.

2. Methods

This is a 104-week open-label extension study of a previous 16-week double blind, randomised, placebo-controlled trial followed by a 40-week open label phase. The patients included in the previous trial have been periodically evaluated at the Centre for Ageing Brain and Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Italy up to two-years. We considered 9 patients out of 10 with the *ELOVL5* c.689G > T (p.Gly230Val) variant, as one died during the study (death not related to the disease). Mean age was 48.7 ± 10.8, and the mean age at onset was 38.4 ± 6.8; six patients were females.

The study was approved by the ethics committee of the Brescia Hospital, Italy (NP1821) and was conformed to the Declaration of Helsinki principles. Written informed consent was obtained from all patients. Trial was registered at ClinicalTrials.gov (NCT03109626). As primary efficacy measure, we considered the mean change from enrolment/baseline to the end point of clinical symptoms, measured by standardised scales. Secondary efficacy measures included changes from baseline to the end point of brain FDG-PET imaging and serum *ELOVL5* levels and DHA levels.

During this 104-week extension, each patient received 600 mg/day b.i.d. of a fish oil derived DHA (Sofedus srl, Milan, Italy). Inclusion and exclusion criteria were previously published [2].

Each patient underwent standardised clinical assessment and blood sampling for evaluation of *ELOVL5* expression by quantitative real-time PCR (see Manes et al. for details) [2] and for dosage of serum DHA levels at 62 week (T1), 82 week (T2) and 104 week (T3) DHA supplementation follow-up, as compared to baseline (T0); moreover, we assessed cerebellar metabolism and neurophysiological parameters at T3, as compared to T0 (see Fig. 1, study design).

Standardised clinical assessment included Scale for the Assessment and Rating of Ataxia (SARA, range 0–40) [5] and the International Cooperative Ataxia Rating Scale, (ICARS, range 0–100) [6]. As this was an open label study, to ensure blindness in the clinical assessment scoring, at each time-point (T0, T1, T2 and T3) neurological examination was video-recorded and analysed in blindness by AA, who was unaware of time-point, as the videos were presented randomly.

Cerebellar metabolism was assessed by brain 18-fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) scan, as previously reported [2]. Briefly, processing and statistical analyses were carried out running on MATLAB (http://it.mathworks.com/products/matlab/) (Mathworks Inc., Sherborn, Mass., USA) and Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm/software/SPM12/), a fully automated, unbiased and operator independent software. Cerebellar metabolism changes were evaluated by non-parametric permutation test (10,000 permutation, Statistical nonParametric Mapping 13, http://warwick.ac.uk/snpm; T0 vs. T3), and the threshold set at $p < 0.05$, Family-Wise Error (FWE) cluster-level corrected [7].

Nerve conduction study (NCS) and Electromyography (EMG) were performed according to standard procedures.

To assess the effect of DHA treatment over time, we applied 1-way repeated measures ANOVA with TIME as within-subjects factors. Mauchly's test was used to test for assumption of sphericity, while Greenhouse–Geisser epsilon determination was used to correct in case of sphericity violation. Results were expressed as mean values ± standard error (SE), unless otherwise specified. Statistical analyses were performed using SPSS version 21 (SPSS, Inc., Chicago, IL, USA). SARA and ICARS scores were normally distributed at each time-point as assessed by Shapiro-Wilk's test ($p > 0.05$). There were no outliers assessed by examination of studentized residuals for values grater ± 3 standard deviations.

3. Results

Nine patients completed the 104-week follow-up. No discontinuations occurred, and no side effects were reported during DHA supplementation at established dosage.

We found a significant maintenance of improvement of clinical symptoms over time. Repeated measures ANOVA performed on total SARA score revealed a main effect of time ($F(3,24) = 9.82, p < 0.001$, partial $\eta^2 = 0.55$). Post-hoc analysis showed a significant reduction of SARA score values at T1 (6.0 ± 1.4, $p = 0.001$), T2 (6.4 ± 1.8, $p = 0.01$) and T3 (6.4 ± 1.8, $p = 0.013$) compared to baseline (8.9 ± 1.4). No significant differences among T1, T2 and T3 time-points were reported.

The same main effect of time was shown for ICARS score ($F(1.35,10.86) = 8.07$, $p = 0.012$, partial $\eta^2 = 0.50$). Post-hoc analysis showed a significant reduction of ICARS score values at T1 (13.6 ± 3.9, $p = 0.008$), T2 (13.1 ± 4.0, $p = 0.012$) and T3 (13.8 ± 4.3, $p < 0.001$) compared to baseline (18.7 ± 4.3). Therefore, we analysed the four sub-items of ICARS score separately. We found a significant amelioration of posture and gait scores at T1 (6.7 ± 2.2, p = 0.021), T2 (6.5 ± 2.2, p = 0.011) and T3 (6.5 ± 2.2, p = 0.003) as compared to baseline (9.0 ± 1.8), as well as a significant amelioration of kinetic scores at T1 (3.1 ± 1.1, p = 0.008), T2 (2.5 ± 1.1, p = 0.013) and T3 (3.8 ± 1.6, p = 0.018) compared to baseline (6.0 ± 1.7). No significant differences among T1, T2 and T3 time-points were reported.

Indeed, six subjects improved in both scales, three subjects only improved in ICARS scale at T3 as compared to baseline (see Fig. 2**, panel A).**

A significant difference in cerebellar metabolism between T0 and T3 was observed, with a sustained significant increase of cerebellar metabolism at T3 as compared to T0 in the left exterior cerebellar lobe (x,y,z = −44, −60, −26; T = 10,26; $p = 0.013$, cluster size = 227) (see Fig. 2**, panel B**). Importantly, no significant differences in the opposite contrast (T0 > T3) were found at the pre-established

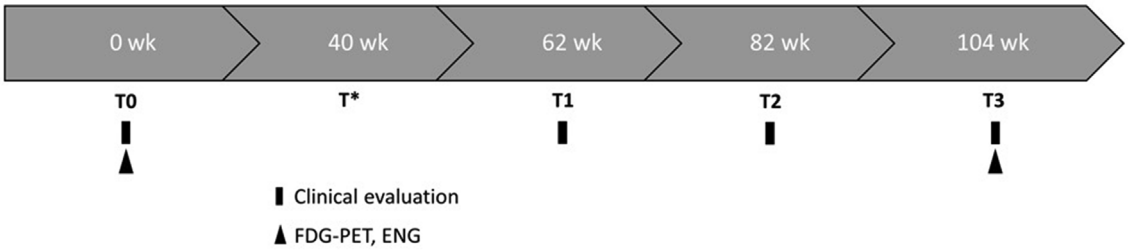

Fig. 1. Study design of the open-label extension study with DHA supplementation in SCA38. Black blocks indicate the time points of clinical assessment; black arrows indicated the time points of brain 18-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and electroneurography (ENG). T0: baseline; T1: 62 weeks follow-up; T2: 82 weeks follow-up; T3: 104 weeks follow-up. T*: previously published open label follow-up study (Manes et al., 2017) at 40 weeks follow-up.wk: weeks.

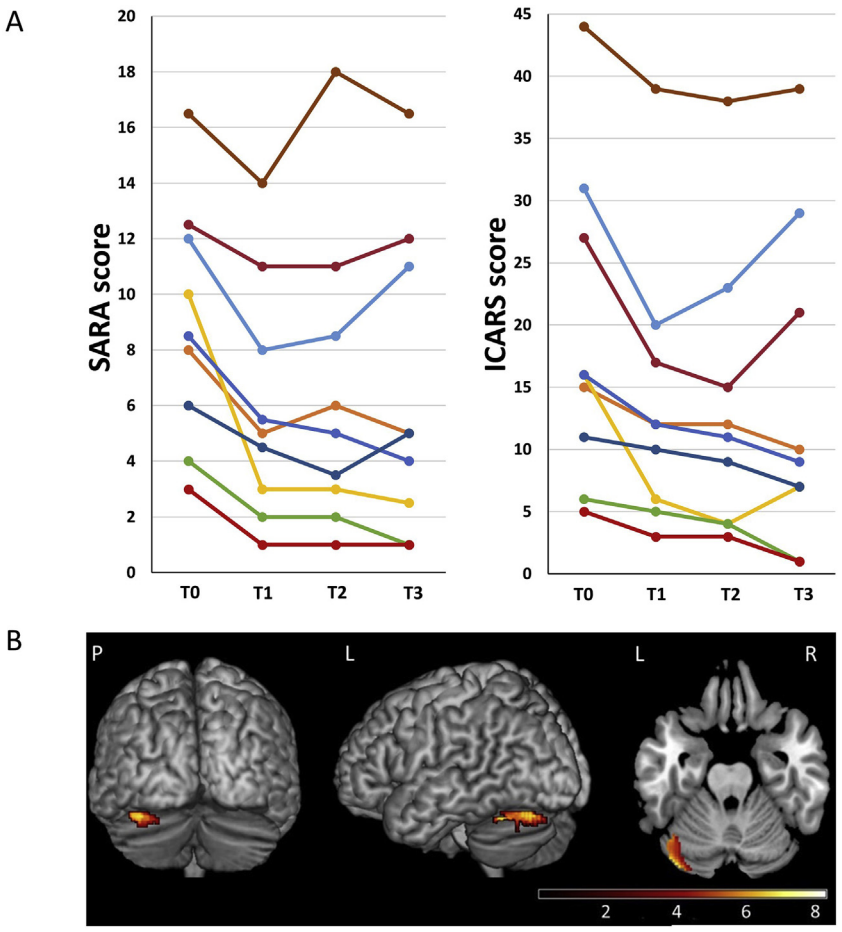

Fig. 2. Clinical assessment and cerebellar metabolism in the open-label extension study. Panel A. SARA and ICARS scores of each patient at each time point. Each patient is reporetd with different coloured line. T0: baseline; T1: 62 weeks follow-up; T2: 82 weeks follow-up; T3: 104 weeks follow-up. See Results section for mean, standard error and p-values of whole group. **Panel B.** Pattern of the mean (average of the 9 patients included in the study) cerebellar metabolism in SCA38 patients at T0 vs. T3 (T0 < T3) ($p < 0.05$, FWE corrected). Only the cluster surviving the statistical threshold is shown. See results for details. The results are superimposed on a 3D standardised template template. P: posterior, L: left; R: right.

threshold, ruling out worsening of regional cerebellar metabolism after 104-week DHA treatment.

At NCS, motor and sensory conduction velocities did not significantly worsen during 104-week DHA treatment in the considered nerves. For instance, the conduction velocities of the right Peroneal nerve at T3 (43.2 ± 0.7, p = 0.780) were comparable to those at baseline (43.3 ± 0.8). Similar results were appreciated for the left Peroneal nerve. Mean sensory velocities of the right Sural nerve (51.2 ± 1.4, p = 0.435) were comparable to baseline (50.0 ± 0.6), and similar results were appreciated also for the right Sural nerve.

No main effect of time in reduction of serum DHA levels was found [F(1.7, 13.6) = 0.56, p = 0.55, partial η2 = 0.57]. We indeed found a slight but not significant increase of total serum DHA levels at T1 (13.4 ± 4.5, p = 0.67), T2 (19.5 ± 21.2, p = 0.31) and T3 (20.8 ± 26.7, $p = 0.41$), compared to baseline (12.0 ± 8.1).

We failed to show a significant main effect of time in reduction of ELOVL5 expression in blood [F(1.85,15.0) = 0.84, p = 0.45, partial η2 = 0.62]. Post-hoc analysis showed no significant reduction of ELOVL5 expression in blood at T1 (0.99 ± 0.29, p = 0.55), T2 (0.95 ± 0.22, p = 0.32) and T3 (1.15 ± 0.24, p = 0.75), as compared to baseline (1.06 ± 0.25).

4. Discussion

In a double-blind, randomised, placebo-controlled study followed by a short-term open label phase, we have previously demonstrated that oral DHA is a safe and effective treatment for SCA38 patients [2]. However, we did not know whether the beneficial effect of this treatment might be extended over time, with no long-term side effects and with no concomitant tolerance mechanisms.

In the present study, by a long-term follow-up, evaluating patients up to 2 years, we have demonstrated that oral DHA at a dosage of 600 mg/day is the eligible treatment for SCA38. By a comprehensive assessment, we found that DHA was able to stabilize clinical symptoms, as measured by SARA and ICARS scales and to maintain the significant amelioration of cerebellar metabolism at the FDG-PET. Brain FDG PET allows the *in vivo* study of cerebral glucose metabolism, thus being extensively used to detect metabolic abnormalities in several neurological diseases. In our study, brain FDG PET revealed a significant amelioration of cerebellar metabolism in SCA38 patients undergoing oral DHA supplementation. This result might reflect the functionally and metabolically restoring of cerebellar cortex after DHA exposure. We hypothesised that increased metabolism in this region could be due to neuronal and synaptic activity changes in the Purkinje cells, but additional studies are needed to confirm this issue.

Moreover, we have further proven that DHA supplementation was well tolerated, affordable and easy to administer dietary intervention, without side effect and with a relatively low cost. Conversely, we failed to demonstrate biological correlates of clinical and instrumental findings.

The use of DHA supplementation in SCA38 stemmed from the initial observation that *ELOVL5* mutations lead to an increased amount of the encoded protein with a mislocalization of the aberrant form in the perinuclear area instead of endoplasmic reticulum and by a decrease of its final products, in particular DHA, in patients' serum [3]. Thus, DHA supplementation can act by compensating the decrease of very long chain fatty acids and by lowering ELOVL5 aberrant protein via a transcriptional feedback loop.

In the next future, it would be important to test the DHA administration to mouse model lacking *ELOVL5* gene [4], which recapitulates some aspects of the human disease, and get a better understanding in the molecular correlates of presymptomatic and symptomatic stages,

and potentially be a guide for better clinical trials.

Given the open-label study design, we tried to minimize possible biases in the clinical evaluation scoring, by video-recording and randomising the clinical assessments and making them evaluating by a neurologist who was unaware of the follow-up time-points. Furthermore, we used an unbiased, automated, and operator independent software for analysing imaging data, which allowed us to exclude regional worsening of cerebellar metabolism at 2-year follow-up.

Even though carried out in a small sample of patients, this study suggests that DHA supplementation is an effective treatment approach in SCA38. Its intake for the whole life would be beneficial but now we have no sufficient data to exclude the development of tolerance associated to a prolonged administration. Based on our observation, we might speculate that DHA treatment is even beneficial in the pre-symptomatic stage of the disease to delay disease onset and slow the progression of symptoms.

Author Roles

B.A., and B.B. contributed to the concept and study design. M.M., A.A., D.G.E., B.L., P.E., M.N., P.M.P., P.C., P.B., O.L., C.C., F.M., T.F., C.D., P.A., B.A., B.B. contributed to the data acquisition and analysis. M.M., A.A., B.A., and B.B. drafted the manuscript and figures, and all authors approved the final version.

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Relevant conflicts of interest/financial disclosures

Authors have nothing to disclose.

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