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

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1 **Long-term efficacy of Docosahexaenoic acid (DHA) for Spinocerebellar Ataxia 38**

2 **(SCA38) treatment: an open label extension study**

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34   **Abstract**

35   **Background:** Spinocerebellar Ataxia 38 (SCA38) is caused by *ELOVL5* gene mutation, with significant

36   reduction of serum docosahexaenoic acid (DHA) levels. DHA supplementation has been proven

37   effective at short-term follow-up.

38   **Objective:** To evaluate long-term safety and efficacy of 600 mg/day oral DHA in SCA38 by a 2-year

39   open label extension study.

40   **Methods:** Nine SCA38 patients underwent standardised clinical assessment at 62 (T1), 82 (T2) and

41   104 (T3) weeks, and compared to pre-treatment scores (T0). Brain 18-Fluorodeoxyglucose Positron

42   Emission Tomography and electroneurography were performed at T0 and T3.

43   **Results:** We found a significant maintenance of clinical symptom improvement at each follow-up time-

44   point ($p<0.001$) as compared to T0, a sustained increase of cerebellar metabolism at T3 as compared

45   to T0 ($p=0.013$), and no worsening of neurophysiological parameters. No side effect was recorded.

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

47

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 (Borroni et al.; Manes Ann Neurol).

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

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 (Manes et al).

Here, we report the results of the subsequent long-term open-label extension study, aimed at evaluating the long-term safety and efficacy of oral DHA supplementation (600 mg/day) on clinical disease course, cerebellar metabolism and neurophysiological parameters up to two years follow-up.

3

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).

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 (Manes).

Each patient underwent standardised clinical assessment 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 **Figure 1**, study design).

Standardised clinical assessment included Scale for the Assessment and Rating of Ataxia (SARA, range

0-40)[8] and the International Cooperative Ataxia Rating Scale, (ICARS, range 0-100)[9]. 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 (Manes). 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

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93   test (10,000 permutation, Statistical nonParametric Mapping 13, http://warwick.ac.uk/snpm; T0 vs.

94   T3), and the threshold set at $p<0.05$, Family-Wise Error (FWE) cluster-level corrected[11].

95   Electromyography/electroneurography (EMG/ENG) examination was performed according to

96   standard procedures.

97   To assess the effect of DHA treatment over time, we applied one-way mixed ANOVA with TIME as

98   within-subjects factors. Mauchly's test was used to test for assumption of sphericity, while

99   Greenhouse–Geisser epsilon determination was used to correct in case of sphericity violation. Results

100   were expressed as mean values$\pm$standard error (SE), unless otherwise specified. Statistical analyses

101   were performed using SPSS version 21 (SPSS, Inc., Chicago, IL, USA).

102   Evaluation of *ELOVL5* expression was assessed by quantitative real-time PCR on retrotranscribed

103   mRNA extracted from nine patients' PAX-gene blood at different time points of DHA supplementation

104   (see REFERENCE): 62 week (T1), 82 week (T2) and 104-week (T3). The mean and S.E. was calculated

105   and normalised to baseline (T0). Data analysis was performed by GraphPad using a second degree

106   polynomial regression curve.

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 η^2 =0.55). Post-hoc analysis showed a significant reduction of SARA score values at T1 (6.0$\pm$1.4, p=0.001), T2 (6.4$\pm$1.8, p=0.01) and T3 (6.4$\pm$1.8, p=0.013) compared to baseline (8.9$\pm$1.4) (**Figure 2, panel A**). 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 η^2 =050). Post-hoc analysis showed a significant reduction of ICARS score values at T1 (13.1$\pm$3.9, p=0.008), T2 (13.0$\pm$4.0, p=0.012) and T3 (13.8$\pm$4.3, $p<0.001$) compared to baseline (18.7$\pm$4.3) (**Figure 2, panel A**). No significant differences among T1, T2 and T3 time-points were reported.

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 **Figure 2, panel B**). Importantly, no significant differences in the opposite contrast (T0>T3) were found at the pre-established threshold, ruling out worsening of regional cerebellar metabolism after 104-week DHA treatment.

At EMG/ENG, motor and sensory conduction velocities did not significantly worse during 104-week DHA treatment.

126 We showed a slight but significant reduction of *ELOVL5* expression in blood comparing each time point

127 with baseline, although a high degree of variability was present. An increased expression was also

128 present in all cases at T3 (r^2: 0.8989)(Figure 3).**Discussion**

129 In a double-blind, randomised, placebo-controlled study followed by a short-term open label phase, we

130 have previously demonstrated that oral DHA is a safe and effective treatment for SCA38 patients

131 (Manes). However, we did not know whether the beneficial effect of this treatment might be extended

132 over time, with no long-term side effects and with no tolerance mechanisms occurring.

133 In the present study, by a long-term follow-up, evaluating patients up to 2 years, we have

134 demonstrated that oral DHA at a dosage of 600 mg/day is the eligible treatment for SCA38. By a

135 comprehensive assessment, we reported that DHA was able to stabilize clinical symptoms, as

136 measured by SARA and ICARS scales, to maintain the significant amelioration of cerebellar

137 metabolism, and to avoid the worsening of neurophysiological measures. Moreover, we have further

138 proven that DHA supplementation was well tolerated, affordable and easy to administer dietary

139 intervention, with no side effect and with a relatively low cost.Clinical findings were in line with the

140 slightly decreased expression of *ELOVL5* in patients' blood. We also noted a final increment of *ELOVL*5

141 expression, which might suggest a compensatory mechanism after long-term administration and

142 possibly an adjustment of the therapy if prolonged for many years..

143 The use of DHA supplementation in SCA38 stemmed from the initial observation that *ELOVL5*

144 mutations lead to an increased amount of the encoded protein with a mislocalization of the aberrant

145 form in the perinuclear area instead of endoplasmic reticulum and by a decrease of its final products,

146 in particular DHA, in patients' serum (Di Gregorio et al., 2014). Thus, DHA supplementation can act by

147 compensating the decrease of very long chain fatty acids and by lowering ELOVL5 aberrant protein via

148 a transcriptional feedback loop.

149 Given the open-label study design, we tried to minimize possible biases in the clinical evaluation

150 scoring, by video-recording and randomising the clinical assessments and making them evaluating by

151 a neurologist who was unaware of the follow-up time-points. Furthermore, we used an unbiased,

152 automated, and operator independent software for analysing imaging data, which allowed us to

153 exclude regional worsening of cerebellar metabolism at 2 year follow-up.

154 Even though carried out in a small sample of patients, this study suggests that DHA supplementation is

155 an effective treatment approach in SCA38. Its intake for the whole lifewould be beneficial but now we

156 have no sufficient data to exclude the development of tolerance associated to a prolonged

157 administration. Based on our observation, we might speculate that DHA treatment is even beneficial in

158 the presymptomatic stage of the disease to delay disease onset and slow the progression of symptoms.

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166 **Author Contributions**

167 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.,

168 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.,

169 B.A., and B.B. drafted the manuscript and figures, and all authors approved the final version.

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171 **Potential Conflicts of Interest**

172 The authors do not have any potential conflict of interest.

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240   **Legend to figures.**

241

242   **Figure 1. Study design of the open-label extension study with DHA supplementation in SCA38.**

243   Black blocks indicate the time points of clinical assessment; black arrows indicated the time points of

244   brain 18-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and electroneurography

245   (ENG). T0: baseline; T1: 62 weeks follow-up; T2: 82 weeks follow-up; T3: 104 weeks follow-up. T*:

246   previously published open label follow-up study (Manes et al., 2017) at 40 weeks follow-up.

247   wk: weeks.

248

249   **Figure 2. Clinical assessment and cerebellar metabolism in the open-label extension study.**

250   **Panel A.** SARA and ICARS scores at baseline (T0), after 62 weeks (T1), 82 weeks (T2) and 104 weeks

251   (T3) DHA treatment in SCA38 patients.

252   *p-value<0.05.

253   **Panel B.** Pattern of cerebellar metabolism in SCA38 patients at T0 vs. T3 (T0<T3) (p<0.05, FWE corrected).

254   See results for details. The results are superimposed on a 3D standardized template template.

255   P: posterior, L: left; R: right.

256

257   Figure 3. *ELOVL5* expression analysis in DHA-treated patients' blood. Expression was evaluated by

258   Quantitative real-time PCR on retrotranscribed mRNA extracted from PAX-gene blood at different time points

259   (62 week (T1), 82 week (T2) and 104 week (T3)). *ELOVL5* was normalized against *TBP* gene. The average

260   value and S.E. is shown. Statistical analysis showed the data had a best fit with a second degree polynomial

261   regression curve (r^2= 0.8989), with an initial reduction of expression and a final increase to levels close to

262   baseline.

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