
eAppendix. Supplemental Methods

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.
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Inclusion/Exclusion Criteria:

Inclusion criteria:

- diagnosis of RP by a retinal specialist, typically including:
  - poor night vision, reduced ERG amplitudes, peripheral field constriction
  - characteristic fundus appearance (i.e., pigmentary disturbances and vessel attenuation)
- additional clinical diagnosis of X-linked RP, typically including:
  - male XLRP subjects with early onset of nightblindness (first two decades of life)
  - early onset of field constriction/legal blindness (second or third decade)
  - presence of at least two affected male relatives, absence of male-to-male transmission
  - the patient’s mother expressing characteristics consistent with carrier state
    - typically a slightly reduced ERG amplitude, a slight delay in implicit time
- early stage X-linked disease (minors and young adults from age 7 to 32 years)
- measurable cone ERG amplitudes: patients with >1.0 μV amplitudes to 31-Hz flicker
- both eyes must meet entry criteria (i.e. no cataracts requiring surgery or retinal detachments)
- willing to supply blood samples at 6-month intervals
- media clarity sufficient for fundus photography
- able to return at yearly intervals
- take daily multi-vitamin and randomly assigned study capsules for the 4-year duration
- ≤4 patients from a single pedigree enrolled unless separated by ≥4 degrees of relatedness
- all ethnic/racial groups

Exclusion criteria:

- excessive fish consumption (e.g., cold water fish such as salmon, tuna, sardines) and/or fish oil supplementation (or other oil containing DHA)
- baseline RBC-DHA levels consistent with high dietary DHA intake (above upper confidence interval for 20 normally-sighted male volunteers ages 10-30 years; i.e., >50 μg/ml or 5.0% RBC-DHA)
- metabolic disease that may interfere with fatty acid metabolism or require anti-coagulant medication

Design:

DHAX was a 4-year, single-site, randomized, placebo-controlled, double-masked Phase II clinical trial conducted at the Retina Foundation of the Southwest in Dallas.

Participants and Participant Discontinuation:

The enrollment criteria of age was modified a year after trial commencement (10-30 years to 7-32 years) to account for the limited availability of eligible XLRP patients in the population. Collection of race and ethnicity information was required by the trial sponsor, U.S. Food and Drug Administration, and self-reported by participants or parents. Ethnicity as Hispanic or Latino or not Hispanic or Latino was selected and racial categories included American Indian/Alaskan Native, Asian, Native Hawaiian or Pacific Islander, Black or African American, White, More than one race, Unknown; multiple categories could be chosen. Participants were provided a stipend ($200 per annual visit) and when necessary, expenses for transportation and accommodation were reimbursed.

Reasons for discontinuation and time on trial of drop-outs correspond to Figure 1.

a Placebo group; n=8; 1 Drop-out due to apathy (12 mo); 1 drop-out due to seeing white circles (6 mo); 2 drop-outs lost contact (12 mo, 10 mo); 2 drop-out’s parents were too busy to travel to Dallas (12 mo, 8 mo); 1 drop-out due to depression from continued vision loss (4 mo); 1 drop-out due to floaters and increased sensitivity to light (2 mo).

b Placebo group; n=2; Two participants completing the trial were removed as one had a mutation in the choroideremia gene (CHD; REP-1) and the second had an autosomal recessive RP mutation (homozygous CNGB1 mutation). These mutations were identified after trial completion.

c Placebo group; n=5; 1 Drop-out moved to Iraq; 2 drop-outs due to apathy (15 mo, 19.5 mo); 1 drop-out ran away from home (38 mo); 1 drop-out reported as being “terribly sick and sore when taking capsules” (35 mo).

d DHA group; n=8; 3 Drop-outs lost to follow-up (0 mo, 4 mo, 15 mo); 1 due to apathy and difficulty in travelling to Dallas (12 mo); 1 changed his mind about participating, he consumed no capsules (0 mo); 1 due to apathy (9 mo); 1 due to inconsistent bowel movements (8 mo); 1 due to dehydration and fatigue (5 mo).
Clinical Evaluation:
A comprehensive ophthalmic examination was conducted annually at Texas Retina Associates (from GEF or RS) and included personal and family history, afferent pupil reaction, slit-lamp examination, retina and vitreous examinations, intraocular pressure, and ocular motility. Blood fatty acids were analyzed semi-annually and comprehensive blood chemistry panel conducted annually to assess potential adverse effects. Since the number of capsules assigned was based on bodyweight, height and weight were assessed using a Healthometer model 402KL (Pelstar, McCook, IL). Scale calibration was checked weekly and certification obtained yearly.

ERG Functional Assessment:
Pupils were maximally dilated using a combination of 1.0% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride. Immediately after dilation, one eye was dark-adapted for 45 min using a combination of an opaque black patch (Bernell Corp., Mishawaka, IN) and opaque adhesive occluders (Coverlet Eye Occlusors, Beiersdorf Inc., Cincinnati, OH). Full-field ERGs were obtained with a bipolar contact lens electrode (Burian Allen, Hansen Ophthalmic Development Laboratories, Coralville, IA) and an Espion® system by Diagnosys (Diagnosys LLC., Lowell, MA). Stimuli were calibrated monthly with a UDT Model 40 integrating photometer (United
Detector Technology, Baltimore, MD). Computer-averaged rod responses were elicited from the dark-adapted eye with dim achromatic flashes, (n=20; 0.01 cd•s/m²), maximum (mixed cone-rod) response were elicited by achromatic flashes (n=10; 3.0 cd•s/m²), and cone responses were elicited by 31 Hz flicker (n=200; 3.0 cd•s/m²).

The light-adapted cone ERG amplitude to a 31 Hz flicker stimulus was recorded at baseline and at each annual visit in the eye with the lowest visual acuity (or left eye if acuity was equal in both eyes). Minimum detectable ERG amplitudes were trough-to-peak amplitudes that were twice the noise level and were reliably identified; these minimums were 0.1, 3.0, and 3.0 µV for cone flicker, dark-adapted rods, and maximum amplitudes, respectively. Normal values and lower limits of normal for each amplitude have been published previously and are given in the Table 2 legend.

**Blood Collection and Fatty Acid Analysis:**

Fasting (>8 hrs) blood was drawn into an ethylenediamine tetraacetate-containing tube (6 ml) from an antecubital vein. RBC fatty acids were analyzed by capillary column gas chromatography using flame-ionization detection according to previously detailed procedures. To assess protocol adherence, RBC-DHA levels were assessed in unmasked interim analyses only by the Data and Safety Monitoring Committee and by laboratory personnel after data were locked for statistical analysis at the end of the trial. Volunteers (n=29; range 7-31 years; 15.7±1.2 years) with normal visual function age-matched with DHAX participants provided normative DHA values of 2.91±0.15% of total RBC lipids (mean±SE).

**Statistical Analysis:**

No missing test results were imputed into the statistical analysis of mITT data. Upon trial termination, a preliminary statistical analysis was performed, data was ‘locked’, unmasking performed followed by final statistical analysis. ERG amplitudes (in µV) were converted to log₁₀ to more closely approximate normal distribution. Trial outcomes were analyzed using a repeated measures mixed model regression analysis with restricted maximum likelihood estimation and a compound symmetric covariance matrix. Two-tailed t tests were used to compare overall group differences for demographics, anthropometrics and fatty acids. Regression analysis was used to assess the association between ERG functional outcomes and RBC-DHA levels. SPSS ver. 22 (IBM Corporation, Armonk, NY) and Statistica ver.12 (StatSoft, Inc, Tulsa, OK) were used to perform statistical analyses.

**eReferences**