iLiNS-DYAD-G2 Statistical Analysis Plan: Effect of LNS on Child Buccal Telomere Length at Preschool Follow-Up

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Background and Objectives

Telomeres are the caps at the ends of DNA strands that protect the chromosome. Telomeres shorten each time a cell divides and replicates, until reaching a limit at which point the cell arrests. Thus, telomeres are part of the regulatory system that determines the longevity of cells, and shorter telomeres have recently been suggested as early predictors for onset of disease and earlier mortality. Telomere length in newborns is variable and thought to be influenced by the prenatal environment, including prenatal nutrition.

Between 2009 and 2014, the International Lipid-Based Nutrient Supplements study (iLiNS DYAD-G trial) was conducted in Ghana to examine the efficacy of LNS for preventing malnutrition in pregnant and lactating women and their children. 1320 pregnant women ≤20 weeks gestation were individually randomized to receive daily, one of three treatments: (a) 60 mg iron plus 400 µg folic acid during pregnancy, and a low dose calcium placebo during the first 6 months postpartum, with no supplementation for offspring during infancy (IFA group) or (b) 1-2 RDA of 18 micronutrients during pregnancy and the first 6 months postpartum, with no supplementation for offspring during infancy (MMN group) or (c) small- quantity lipid-based nutrient supplements (20 g) which contained 22 micronutrients plus some macronutrients, during pregnancy and the first 6 months postpartum, with SQ-LNS for offspring from 6 to 18 months (SQ-LNS group). Women were followed until 6 mo postpartum, and their infants until 18 mo of age.

We conducted a follow-up study of the iLiNS DYAD-G cohort when the children reached age 4– 6 y and one of our aims was to examine the long-term impact of exposure to SQ-LNS on buccal telomere length in children at age 4-6 y. Buccal telomere length samples have been analyzed for a subset of children from the SQ-LNS group and the IFA group. For the analysis described in this document, the primary objective is to evaluate the effect of maternal and child supplementation with SQ-LNS on child buccal telomere length at 4-6 years of age.

Hypotheses

1) Children born to women in the SQ-LNS group will have a longer mean buccal telomere length than children born to women in the IFA group.

Description of Variables

Outcome Variable

Buccal telomere length: Buccal telomere length will be analyzed as a continuous variable as well as a dichotomous variable. Those below the median will be identified as short telomere length. Secondarily, we will explore the possibility of defining short telomere length at the 25^{th} or 10^{th}

percentile. Telomere length was determined in lab analyses by comparing the amount of the telomere amplification product (T) to that of a single-copy gene (S). Telomere length will be presented as a T/S ratio, which is consistent with telomere length literature.

Covariates

All models will include child's age since not all children were measured during the follow-up at the same age. The following variables will be considered as covariates:

- Child sex
- Maternal age
- Maternal education
- Gestational age at enrollment
- Maternal parity (nulliparous vs. parous)
- Maternal AGP at enrollment
- Maternal CRP at enrollment
- Maternal BMI at enrollment adjusted for gestational age
- Season at enrollment

Each variable that shows a statistically significant association with telomere length ($P<0.1$) will be included in the model.

Effect Modifiers

The following variables will be considered as significant effect modifiers at $p < 0.10$:

- Child sex
- Maternal age
- Maternal parity (nulliparous vs. parous)
- Gestational age at enrollment
- Maternal AGP
- Maternal CRP

Statistical Analysis Methods

Framework

All hypothesis testing will be two-sided and testing will be considered significant at the 0.05 unless otherwise specified.

Data Cleaning

Data collected during the main trial have been cleaned previously. The majority of data cleaning for iLiNS-DYAD-G2 occurred concurrently during data collection, with queries identified using SAS and relayed to the local home visit team manager. Queries were then resolved by seeking clarification from the field worker who completed the form and/or by re-contacting the

respondent. Data cleaning will be double checked before beginning analysis by producing stem-and-leaf plots, histograms and/or scatterplots of variables.

Outliers

Outliers will be identified by visually inspecting histograms and/or scatterplots of variables. Outliers that are clearly impossible or implausible values will be corrected if possible and otherwise recoded as missing. Outliers which are plausible or possible will be retained. In cases where extreme outliers are retained, sensitivity analysis will be performed to determine whether the extreme outliers have undue influence on the results.

Data transformation

We will inspect the residuals of telomere length after model fitting for normality and transform as necessary. If no suitable transformation is found, ranks will be calculated or categories will be used for analysis.

Model Assumptions

Models will be checked to ensure that the residuals are normally distributed and the heteroscedasticity assumption is met through the residual versus fit plot.

Software

Data will be analyzed using SAS version 9.4.

Analysis of the Effect of the Intervention

The analysis will be by intent-to-treat. That is, by-group analysis will be according to group assignment regardless of any protocol violations. Secondarily, two per protocol analyses will be performed based on self-reported adherence to supplementation during the main trial. The first per protocol analysis will include women with ≥ 80% adherence to supplement during pregnancy. The second per protocol analysis will include women with ≥80% adherence during the entire period of pregnancy up to 6 months postpartum. This SAP does not need to address the issue of the supplement switch that occurred during the main trial between IFA and MMN since the subset selected is limited to women that either received SQ-LNS or received IFA.

Difference between groups will first be tested in minimally adjusted models, only controlling for child age and then repeated in adjusted models controlling for covariates selected in bivariate models as described above.

For the continuous outcome variable (mean telomere length), the difference between the two groups will be tested with ANCOVA and null-hypothesis of no difference between groups will be rejected if $p < 0.05$. For dichotomous outcome variables, global null hypothesis of no differences between groups will be tested with logistic regression.