ISRCTN14266771 https://doi.org/10.1186/ISRCTN14266771

Epigenetic mechanisms linking a mothers' nutrition to the health of her children in India and Sub-Saharan Africa

Submission date
08/05/2017

Recruitment status
No longer recruiting

Registration date
18/05/2017

Overall study status
Completed

Last Edited
15/06/2023

Condition category
Nutritional, Metabolic, Endocrine

[_] Prospectively registered
[X] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
Maternal nutrition before and during pregnancy is very important and can impact the health of the baby. This study, known as the EMPHASIS study, is a joint initiative between research institutes in the UK, India and The Gambia, established to investigate how maternal nutrition before and during pregnancy could influence offspring health. This study brings together two previous randomized controlled trials investigating the effectiveness of maternal dietary intervention on fetal development: the Mumbai Maternal Nutrition Project (MMNP) in India (ISRCTN62811278) and the Peri-conceptional Multiple Micronutrient Supplementation Trial (PMMST) in rural Gambia (ISRCTN13687662). EMPHASIS extends this work to characterize DNA methylation, an epigenetic mechanism involved in the how genes are expressed, in the children of these mothers, and link this to later life health outcomes. The aim of the study is to provide better advice about nutrition to young women planning their pregnancies and working towards the improvement of maternal and child health and inter-generational prevention of non-communicable chronic diseases.

Who can participate?
Children aged five to seven whose mothers took part in MMNP in India and children aged seven to nine whose mothers took part of the PMMST in the Gambia.

What does the study involve?
In the MMNP study, participants were given nutrient rich food supplements before and during pregnancy. In the PMMST study, participants were given a nutritional supplement (UNICEF/WHO multiple micronutrient preparation (UNIMMAP) until they were pregnant. The children of these women are followed up at ages five to seven (MMNP) and seven to nine (PMMST) for data on their growth, body composition, cardio-metabolic risk markers (chance of having diabetes, heart disease or stroke) and cognitive (mental) function. They also provide DNA samples through a cheek swap as well as blood samples. The DNA samples for both groups of children are analysed to see if maternal nutritional supplements influenced DNA methylation.

What are the possible benefits and risks of participating?
There are no notable benefits or risks with participating.

Where is the study run from?
The study is being run by the University of Southampton (UK) and takes place in rural Gambia and Mumbai, India. Laboratory analysis of DNA methylation is performed at CSIR Centre for cellular and Molecular Biology, Hyderabad, India. Data analysis takes place in all participating centres.

When is the study starting and how long is it expected to run for?
May 2015 to May 2020

Who is funding the study?
1. Medical Research Council and Department for International Development UK, via the Newton Fund (UK)
2. Department of Biotechnology, Government of India (India)

Who is the main contact?
Professor Caroline Fall
chdf@mrc.soton.ac.uk

Study website
http://www.emphasisstudy.org

Contact information

Type(s)
Public

Contact name
Prof Caroline Fall

ORCID ID
http://orcid.org/0000-0003-4402-5552

Contact details
MRC Lifecourse Epidemiology Unit
University of Southampton
Southampton General Hospital
Tremona Road
Southampton
United Kingdom
SO16 6YD
+44 (0)2380 777624
chdf@mrc.soton.ac.uk

Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers
EMPHASIS1

Study information

Scientific Title
Epigenetic Mechanisms linking Pre-conceptional nutrition and Health Assessed in India and Sub-Saharan Africa

Acronym
EMPHASIS

Study objectives
1. Maternal nutritional supplementation around the time of conception and/or in pregnancy influences DNA methylation in her children
2. DNA methylation, either related or unrelated to maternal nutritional supplementation, is associated with phenotypic characteristics in the children (including growth and body composition, cardio-metabolic risk markers, cognitive function and bone mineral content and density).

Ethics approval required
Old ethics approval format

Ethics approval(s)
1. Intersystem Biomedica Ethics Committee, Mumbai, 31/05/2013, ref: ISBEC/NR-54/KM/JVJ /2013
2. Gambia Government / MRC Unit The Gambia's Ethics Committee, 19/10/2015, ref: SCC 1441

Study design
Observational cohort study

Primary study design
Observational

Secondary study design
Cohort study

Study setting(s)
Not specified

Study type(s)
Not Specified

Participant information sheet
Not available in web format, please use the contact details below to request a patient information sheet

Health condition(s) or problem(s) studied
DNA methylation, growth, body composition, neuro-development, cardio-metabolic risk, bone health

Interventions
The EMPHASIS study is an observational study of DNA methylation in two cohorts of children who were born to mothers who took part in two separate completed randomised trials of nutritional interventions before and during pregnancy in Mumbai and in the Gambia.

In the Mumbai trial, (www.isrctn.com/ISRCTN62811278) participating mothers were given a daily snack containing micronutrient-rich foods (green leafy vegetables, fruit and milk) in addition to their normal diet from before pregnancy until delivery. Control women received snacks made from foods of low micronutrient content. The primary outcome was birth weight. The children have been followed up annually since birth for anthropometry, and have been taking part in a follow-up study ("SARAS KIDS") in which their anthropometry, body composition and skeletal development (DXA), cardio-metabolic risk markers (blood pressure, serum lipids, plasma glucose and insulin values during an oral glucose tolerance test) and cognitive function are measured between the ages of five to seven years. Whole venous blood samples, collected by venepuncture after application of EMLA anaesthetic cream, and buccal swabs, obtained by gently scraping the mucosa inside the cheek, are collected for DNA. The DNA from these samples collected during SARAS KIDS are analysed in the EMPHASIS study.

In the Gambian trial, (www.isrctn.com/ISRCTN13687662) participating mothers were given a daily film-coated tablet containing approximately one Reference Nutrient Intake of a range of micronutrients (UNICEF/WHO/United Nations multiple micronutrient preparation (UNIMMAP)) from before pregnancy until the diagnosis of pregnancy (approximately 12 weeks gestation). The control group received a placebo tablet. The primary outcomes were ultrasound indices of utero-placental vascular-endothelial function in mid-gestation. The children were followed up during infancy, and are re-traced and recruited for the EMPHASIS study at the age of seven to nine years. The investigations and samples collected are harmonised with the Mumbai children; DNA samples have been collected, and anthropometry, body composition, cardio-metabolic risk markers and cognitive function have been measured using similar methods.

A full analysis plan is available on the EMPASIS study website (www.emphasisstudy.org). DNA methylation is measured in a single laboratory (Hyderabad, India). Data from the two cohorts will be analysed separately, in two stages: intervention-methylation effects and methylation-phenotype associations. In stage 1, after pre-processing of the raw data, quality control and normalization procedures, intervention methylation effects will be analysed by comparing methylation data at >800,000 CpG sites from the EPIC arrays between trial allocation groups, to identify differentially methylated positions (DMPs) and regions (DMRs) controlling for the false discovery rate (FDR) <5%, and variably methylated regions (VMRs). Technical validation of DMPs and DMRs will be performed by pyrosequencing in a subset of samples. A small number of candidate loci not present on the EPIC array, selected a priori for evidence of association with maternal nutritional exposures and/or health outcomes of interest in other studies, will also be assayed in both cohorts by pyrosequencing. Replication of technically validated loci will be performed using pyrosequencing in an independent sample (Mumbai samples only). Statistical power calculations indicate the ability to detect mean differences in methylation of 3% and 5% in the Indian and Gambian cohorts respectively. In a cross-tissue analysis, technically validated significant loci will be examined in buccal DNA samples (n~50 from each cohort). In stage 2, significant loci associated with the nutritional intervention in either cohort from the EPIC array analysis and all candidate loci will be tested for associations with phenotype outcomes

measured in the children. Loci identified in a meta-analysis of Stage 1 associations across both cohorts will be considered. A ‘hypothesis-free’ analysis to identify loci where methylation is associated with outcomes, irrespective of intervention, will also be performed. All samples will be genotyped using the Illumina GSA array. To explore underlying metabolic mechanisms, gene pathways analysis will be performed for the intervention-outcome GWAS. SNP effects on methylation will be explored through methylation Quantitative Trait Loci (mQTL) analysis and causal analysis using Mendelian randomisation.

Intervention Type
Other

Primary outcome measure
The effects of maternal supplementation on the children's DNA methylation:
1. Epigenome wide DNA methylation is measured in the children’s blood DNA samples using Illumina Infinium Methylation EPIC arrays at age 5-7 y (India) and 7-9 y (The Gambia)
2. Locus-specific DNA methylation is measured in blood and buccal DNA samples using pyrosequencing on Pyromark 96 at age 5-7 y (India) and 7-9 y (The Gambia)

The associations of DNA methylation with phenotype in the children:
1. Birth weight and length are measured using digital weighing scales and a neonatal stadiometer respectively, within 10 days (India) or 72 hours (The Gambia) of birth. Smallness for gestational age (SGA) is calculated from measured birth weight using the INTERGROWTH reference.
2. Children’s weight and height are measured using standardised anthropometric methods (body mass index is calculated from these measures) at age 5-7 y (India) and 7-9 y (The Gambia)
3. Children’s total and regional lean mass, fat mass and body fat percent are measured using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy in India, Lunar iDXA in The Gambia) (lean mass index and fat mass index are calculated from these measures) at age 5-7 y (India) and 7-9 y (The Gambia)
4. Children’s fasting plasma glucose concentration, and 30-and 120-minute plasma glucose concentrations after an oral glucose load, are measured using standard enzymic assays by autoanalyser at age 5-7 y (India) and 7-9 y (The Gambia)
5. Children’s fasting and 30-minute plasma insulin concentrations are measured using Mercodia ELISA on a Victor 2 analyser (India) and an SM-chemiluminescence method (The Gambia) at age 5-7 y (India) and 7-9 y (The Gambia). Insulin resistance is calculated from fasting glucose and insulin values using the HOMA-IR Oxford online calculator. Disposition index is calculated from fasting and 30-minute plasma glucose and insulin values using standard formulae.
6. Children’s resting systolic blood pressures is measured using an OMRON automated blood pressure device, after at least 5 minutes seated at rest, at age 5-7 y (India) and 7-9 y (The Gambia)
7. Children’s fasting plasma or serum total, LDL- and HDL-cholesterol, and triglycerides are measured using standard Enzymic assays on an autoanalyser at age 5-7 y (India) and 7-9 y (The Gambia)
8. Children’s cognitive ability is assessed using the raw and combined scores from cognitive tests from the Kaufmann Assessment Battery for Children (Atlantis, pattern reasoning and word order) and from the Wechsler Intelligence Scale for Children (verbal fluency, Kohs block design and coding) at age 5-7 y (India) and 7-9 y (The Gambia)
9. Children’s bone mineral content, bone area and bone mineral apparent density are measured using DXA at age 5-7 y (India) and 7-9 y (The Gambia)

Secondary outcome measures

The associations of DNA methylation with phenotype in the children:
1. Newborn head, chest, abdominal and mid-upper-arm circumferences are measured using anthropometric tape within 10 days (India) or 72 hours (The Gambia) of birth
2. Newborn triceps and subscapular skinfolds are measured using Holtain skinfold calipers within 10 days (India) or 72 hours (The Gambia) of birth
3. Newborn gestational age at birth was derived from the mother's last menstrual period (LMP) date and early pregnancy fetal ultrasound measurements, both recorded prospectively by the research team. The number and percentage of pre-term newborns (<37 weeks completed gestation) are derived using gestational age.
4. Newborn low birth weight (<2500g) is derived from measured birth weight
5. Children's sitting height, leg length, head circumference, mid-upper arm circumference, chest circumference, waist circumference, hip circumference, and skinfolds are measured using standardised anthropometric methods at age 5-7 y (India) and 7-9 y (The Gambia). Sitting height /leg length ratio, sum of skinfolds and waist/hip ratio are calculated from these measures. Longitudinal indices of growth are derived from these measurements and weight, height and body mass index, using conditional and other modelling techniques.
6. The number and percentage of children stunted, wasted, and underweight are derived from measured weight and height at age 5-7 y (India) and 7-9 y (The Gambia) using the World Health Organization/ Centers for Disease Control child growth reference (www.who.int/childgrowth/en /)
7. Children's android and gynoid fat mass are measured using DXA at age 5-7 y (India) and 7-9 y (The Gambia)
8. Children's resting diastolic blood pressures is measured using an OMRON automated blood pressure device, after at least 5 minutes seated at rest at age 5-7 y (India) and 7-9 y (The Gambia)
9. Children's plasma insulin concentration 30-minutes after an oral glucose load is measured using Mercodia ELISA on a Victor 2 analyser (India) and an SM-chemiluminescence method (The Gambia) at age 5-7 y (India) and 7-9 y (The Gambia). Insulinogenic index is calculated from fasting and 30-minute plasma insulin and glucose values using a standard formula.
10. Metabolic Syndrome is derived from fat mass (DXA), blood pressures, and plasma glucose, insulin, HDL-cholesterol and triglyceride measurements at age 5-7 y (India) and 7-9 y (The Gambia) using values above the age-, sex-, and site-specific upper quartiles for each
11. A category of 'high blood pressure' is derived using the systolic and diastolic blood pressure values and reference data at age 5-7 y (India) and 7-9 y (The Gambia)
12. Children's tibial total and trabecular volumetric bone density and bone area, and diaphyseal bone area, cortical area, thickness, bone mineral content, cortical volumetric bone mineral density and strength (cross-sectional moment of inertia) are measured (in the Gambian children only) using pQCT (Peripheral quantitative computed tomography) at age 7-9 y

Overall study start date
28/05/2015

Completion date
28/05/2020

Eligibility

Key inclusion criteria
1. Healthy children aged five to seven years participating in the SARAS KIDS study (2013-2018) who were born as singleton live births to mothers who took part before and during pregnancy in the Mumbai Maternal Nutrition Project in India (http://www.isrctn.com/ISRCTN62811278) and

who started supplementation at least 3 months prior to conception
2. Healthy children aged five to seven years in the Gambia who were born as singleton live births to mothers who took part in the Peri-conceptional Multiple Micronutrient Supplementation Trial (http://www.isrctn.com/ISRCTN13687662) in the Gambia
3. Parents consented to their participation in follow-up studies in which size, body composition, cardio-metabolic risk markers, cognitive function, and bone health were measured
4. Consented to the use of their DNA samples for this research
5. Samples contained sufficient DNA

Participant type(s)
Healthy volunteer

Age group
Child

Lower age limit
5 Years

Upper age limit
7 Years

Sex
Both

Target number of participants
SARAS KIDS aims to investigate as many as possible of the 1,962 children born as singleton live births to women participating in the original MNP trial in India. EMPHASIS will include those born to women who started supplementation at least three months prior to conception (approximately 900-1100). Their data collection is ongoing, and is expected to be completed in May 2018. In the Gambia, 298 children will be included; these are all the children who were re-traced at age 7-9 years, whose parents consented to their participation, and who took part in the study, out of 356 singleton live births in the original PMMST trial. Their data collection was completed in 2016-2017. 293 had adequate DNA samples for the EMPHASIS study analysis.

Key exclusion criteria
Children whose mothers took part in the Mumbai trial but who did not start supplementation at least 3 months before conception.

Date of first enrolment
04/12/2015

Date of final enrolment
31/07/2018

Locations

Countries of recruitment
England

Gambia

India

United Kingdom

Study participating centre
MRC Lifecourse Epidemiology Unit
University of Southampton
Southampton General Hospital
Tremona Road
Southampton
United Kingdom
SO16 6YD

Study participating centre
CSIR Centre for Cellular and Molecular Biology
Habsiguda, Uppal Road
Andhra Pradesh
Hyderabad
India
500 007

Study participating centre
Centre for the Study of Social Change
M N Roy Human Development Campus
Opp Govt. Colony Building
No. 326
Bandra (E)
Maharashtra
Mumbai
India
400 051

Study participating centre
MRC Unit, The Gambia
Atlantic Boulevard
Fajara
Banjul
Gambia
P. O. Box 273

Study participating centre

London School of Hygiene and Tropical Medicine
Keppel Street
London
United Kingdom
WC1E 7HT

Study participating centre
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge
United Kingdom
CB1 9NL

Sponsor information

Organisation
University of Southampton

Sponsor details
University Road
Southampton
England
United Kingdom
SO17 1BJ

Sponsor type
University/education

Website
http://www.southampton.ac.uk

ROR
https://ror.org/01ryk1543

Funder(s)

Funder type
Government

Funder Name
Medical Research Council and Department for International Development UK, via the Newton Fund

Funder Name
Department of Biotechnology , Ministry of Science and Technology

Alternative Name(s)
Dept. of Biotechnology, Govt of India, , , Department of Biotechnology, Department of Biotechnology, Ministry of Science & Technology, India, Department of Biotechnology, GOI, Dept. of Biotechnology, Govt. of India, Department of Biotechnology, Ministry of Sc & Tech, Govt of India, DBT

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
India

Results and Publications

Publication and dissemination plan
The EMPHASIS analysis plan is available on the EMPHASIS website (www.emphasisstudy.org). A protocol paper, for an open-access peer-reviewed journal is in preparation, and is expected to be published in 2017. The results of the main EMPHASIS analyses (relating maternal nutritional supplementation to DNA methylation, and relating DNA methylation to phenotypic characteristics in the children) are expected to be presented at conferences and published in a series of papers in peer-reviewed journals in 2018 and 2019. Planned subsequent publications, exploring evidence for causality of any associations between DNA methylation and phenotype, using Mendelian randomisation methods.

Intention to publish date
31/12/2019

Individual participant data (IPD) sharing plan
The current data sharing plans for the current study are unknown and will be made available at a later date.

IPD sharing plan summary
Data sharing statement to be made available at a later date

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Protocol article	protocol	30/10/2017	30/06/2020	Yes	No
Results article	results	01/10/2020	08/09/2020	Yes	No

Results article	09/01/2022	15/06/2023	Yes	No