Received: 20 April 2021 | Revised: 15 September 2021 | Accepted: 19 September 2021

DOI: 10.1111/ppe.12826

STUDY DESIGN

Paediatric and Perinatal Epidemiology WILEY

The Nutrition in Early Life and Asthma (NELA) birth cohort study: Rationale, design, and methods

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Abstract

Background: Primary prevention strategies for asthma are lacking. Its inception probably starts in utero and/or during the early postnatal period as the developmental origins of health and disease (DOHaD) paradigm suggests.

Objectives: The main objective of Nutrition in Early Life and Asthma (NELA) cohort study is to unravel whether the following factors contribute causally to the developmental origins of asthma: (1) maternal obesity/adiposity and foetal growth; (2) maternal and child nutrition; (3) outdoor air pollution; (4) endocrine disruptors; and (5) maternal psychological stress. Maternal and offspring biological samples are used to assess changes in offspring microbiome, immune system, epigenome and volatilome as potential mechanisms influencing disease susceptibility.

Population: Randomly selected pregnant women from three health areas of Murcia, a south-eastern Mediterranean region of Spain, who fulfilled the inclusion criteria were invited to participate at the time of the follow-up visit for routine foetal anatomy scan at 19–22 weeks of gestation, at the Maternal-Fetal Medicine Unit of the "Virgen de la Arrixaca" University Clinical Hospital over a 36-month period, from March 2015 to April 2018.

Design: Prospective, population-based, maternal-child, birth cohort study.

Methods: Questionnaires on exposures and outcome variables were administered to mothers at 20–24 gestation week; 32–36 gestation week; and delivery. Children were

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Funding information
We acknowledge all funding sources for the NELA birth cohort study: Instituto de Salud Carlos III, Spanish Ministry of Science, Innovation and Universities (Grants: MS14/00046; CP14/00046; PIE15/00051; PI16/00422; FI17/00086; PI19/00863; FPU18/00545), Fondos FEDER, Fundación Séneca, Agencia de Ciencia y Tecnología Región de Murcia (Grant: 20877/PI/18). Funders have no influence of any kind on analyses or results interpretations

surveyed at birth, 3 and 18 months of age and currently at 5 years. Furthermore, physical examinations were performed; and different measurements and biological samples were obtained at these time points.

Preliminary results: Among the 1350 women invited to participate, 738 (54%) were finally enrolled in the study and 720 of their children were eligible at birth. The adherence was high with 612 children (83%) attending the 3 months' visit and 532 children (72%) attending the 18 months' visit.

Conclusion: The NELA cohort will add original and unique knowledge to the developmental origins of asthma.

KEYWORDS
allergy, asthma, diet, mother-child cohort, wheezing

1 | BACKGROUND

The early life programming concept was originally proposed to explain the link between maternal nutritional deficits during pregnancy, low birth weight and associated risks for obesity, diabetes and cardiovascular disease in lifespan.[1] These initial observations were the basis of the developmental origins of health and disease (DOHaD) paradigm.[2,3] Population-based prospective pregnancy and birth cohorts provide one of the most appropriate study designs to investigate early life hazards at critical time points of prenatal and postnatal human development.

According to the Global Asthma Report 2018, asthma affects over 330 million people worldwide and its prevalence is rising.[4] Moreover, asthma is the most common chronic disease among children.[5] In addition, approximately 30%–40% of the world's population is affected by one or more allergic conditions, with vast personal, social, and economic costs.[6] Despite some advances in treatment, this condition continues to be a public health concern, still killing as many people as malaria.[7] Primary prevention is thus essential to reduce the burden of asthma.

The Nutrition in Early Life and Asthma (NELA) study was launched to try and offer information which may produce primary prevention interventions. The main objective of the NELA study was to investigate whether maternal obesity/adiposity and foetal growth; prenatal and postnatal nutrition; outdoor air pollution; endocrine disruptors; and maternal psychological stress contribute causally to the development of asthma.

2 | METHODS

2.1 | Cohort design and population

The NELA study is a prospective, population-based, maternal-child, birth cohort study which was set up in 2015 at the "Virgen de la Arrixaca" University Clinical Hospital of Murcia, a south-eastern Mediterranean region of Spain. Randomly selected pregnant women who fulfilled the inclusion criteria were invited to participate at the time of the follow-up visit at the aforementioned hospital for routine foetal anatomy scan at 19–22 gestation week, at the Maternal-Fetal Medicine Unit of the hospital from March 2015 to April 2018. During that period, two mornings per week, one of the two principal investigators invited to participate as many mothers as possible from those attending the unit and meeting the inclusion criteria.

Inclusion criteria were: Caucasian and Spanish origin; 18–45 years of age; living in Health Area I (suburban and rural) or in certain districts of Health Areas VI and VII (mainly urban) of the Region of Murcia (Figure 1); planning to live in the area of study during at least 2 years; singleton pregnancy; spontaneous conception; intention to deliver in the aforementioned hospital; and normal ultrasound findings at the time of the visit (no major foetal malformations). Exclusion criteria included: chronic disease in the mother, such as pregestational diabetes mellitus or other major endocrine disorders, pregestational hypertension, autoimmune disease, or cancer; and verbal communication problems.

Synopsis

Study question

- Is asthma programmed during the prenatal and early postnatal developmental periods? If so, what are the key developmental influences and the potential underlying mechanisms?

What's already known

- Some birth cohorts have investigated diverse developmental influences modifying the risk of asthma in the offspring during childhood; however, major gaps in the origins and pathways to asthma persist.

What this study adds

- The NELA study aims to investigate both established and novel developmental influences responsible for asthma and related phenotypes and to decipher the mechanisms and pathways implicated in the long-lasting effects. Results may help to predict the disease and develop strategies for primary prevention, which is the ultimate approach to reducing the burden of NCDs, and the greatest potential for this lies in early life.

2.2 | Data collection and follow-up time points

Mothers had three follow-up visits, one in the second trimester of pregnancy (between 20 and 24 gestation week), one in the third trimester (between 32 and 36 gestation week), and one at delivery. Children have been followed up at birth, and at 3 and 18 months of age so far. They are being followed up at 5 years and will be followed up further on (Figure 2). Table 1 (mothers) and Table 2 (children) show the information obtained or planned at the different visits from mothers and children respectively.

2.3 | Specific objectives

- To investigate whether maternal adiposity and whether foetal growth patterns at 12, 20 and 32 gestation week are associated with lung function and volatile organic compounds in exhaled air (eVOCs) at 3 months of age; and with subsequent asthma.
- To establish whether factors acting in the mother during pregnancy are associated with changes in lung function and/or in pattern(s) of eVOCs at 3 months; and to subsequent asthma.
- To establish whether any of the potential associations is mediated by a change in the Th1/Th2 immunological profile.
- To investigate disturbances in the maternal and offspring microbiome in relation to developmental influences and their role in subsequent occurrence of asthma.
- To try and detect any epigenetic changes in offspring at birth which could mediate the potential effects of the aforementioned factors acting in the mother during pregnancy.
- To build up a unique biobank sample collection with maternal and child biological samples.

2.4 | Main outcome variables

The main outcome variables are lung function at three months of age of the children; and asthma (symptoms and/or diagnosis) and related phenotypes at any point of the follow-up visits (Figure 3).

2.4.1 | Lung function

In those children whose parents authorised the needed sedation for the technique (chloral hydrate 80–100 mg/Kg), lung function tests were performed at the Paediatric Respiratory unit of the hospital. Forced vital capacity (FVC), forced expiratory volume at 0.5 seconds (FEV0.5) and forced expiratory flows at 75 and 25%–75% of FVC

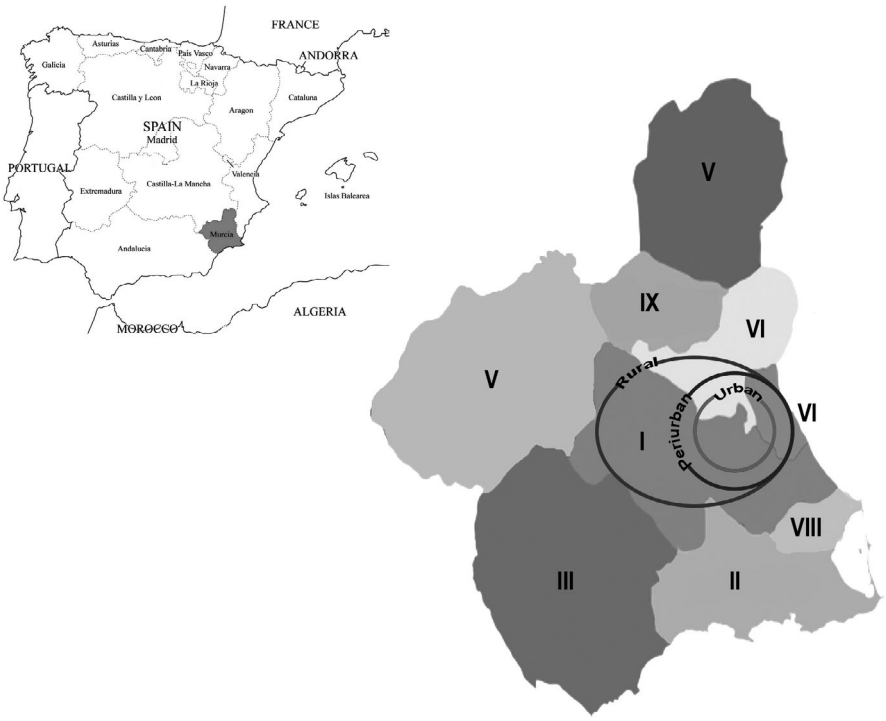

FIGURE 1 Sampling areas of the province of Murcia. The roman numbers indicate heath areas

(FEF75 and FEF25-75) were obtained from maximal expiratory volume curves by means of the raised volume rapid thoracic compression (RVRTC) technique according to the ERS/ATS consensus statement.[8] Oxygen saturation was continuously measured until recovery from sedation. Tests were performed with a Master-Screen BabyBody plethysmograph (Jaeger, Germany) and raised volume was achieved by coupling a Neopuff infant resuscitator (Fisher & Paykel Healthcare, New Zealand) to the facemask. According to published regression formulas corrected for the Jaëger equipment, z-scores of FVC, FEV0.5, FEF75 and FEF25-75 were calculated.[9] Although RVRTC needs sedation and is time-consuming, we preferred it to the multiple breath washout with lung clearance index (MBW-LCI), which can be performed in non-sedated infants because RVRTC measures lung flows and volumes very similarly to spirometry, while MBW-LCI measures ventilation inhomogeneity. This and other lung function techniques, although providing measurements of lung function, are not directly comparable to spirometry.

2.4.2 | Symptoms and diagnoses

Symptoms of potential asthma, allergic rhinoconjunctivitis and atopic eczema and their medical diagnoses by the mothers' or children' doctors were surveyed in all follow-up visits. Symptoms were asked for using questions from the "Estudio Internacional de Sibilancias en Lactantes" (EISL)[10] or Global Asthma Network (GAN)[11] questionnaires, depending on the age, to mothers and children. Questions on wheezing in the different visits will allow to categorise wheezing children into the classical phenotypes of transient early, late-onset and persistent to know whether risk or protective factors vary across phenotypes.

2.5 | Developmental influences

2.5.1 | Maternal adiposity and foetal growth

Maternal abdominal ultrasounds and Doppler examinations (Voluson 730 Expert, GE Medical Systems, Austria) were performed, recorded and used to obtain foetal biometry at 20 and 32 gestation weeks. Foetal weight was estimated from those measurements.[12] Amniotic fluid and placental location and structure were also assessed. Doppler pulsatility index of umbilical and maternal uterine arteries was also measured. Abdominal ultrasound was used to measure subcutaneous fat thickness.[13] Ultrasound scan at 12 gestation week was obtained from clinical records and gestational age was calculated from the foetal crown-rump length.[14]

2.5.2 | Maternal body composition

Maternal body composition throughout pregnancy was measured by bioelectrical impedance (Bodystat Quadscan 4000, Isle of Man, UK). Somatotype was obtained from 10 anthropometric variables: weight, height, skin folds (triceps, subscapularis, supraspinatus and calf) and diameters (arm and calf). Medidep (Vigo, Spain) was used to calculate the percentages of bone, lean and fat mass, together with the components of endomorphy, mesomorphy and ectomorphy.[15] Somatotype will be represented as somatochart.

2.5.3 | Maternal diet during pregnancy

Diet information was collected at 20 gestation week, using a Food Frequency Questionnaire (FFQ) based on a validated questionnaire[16]

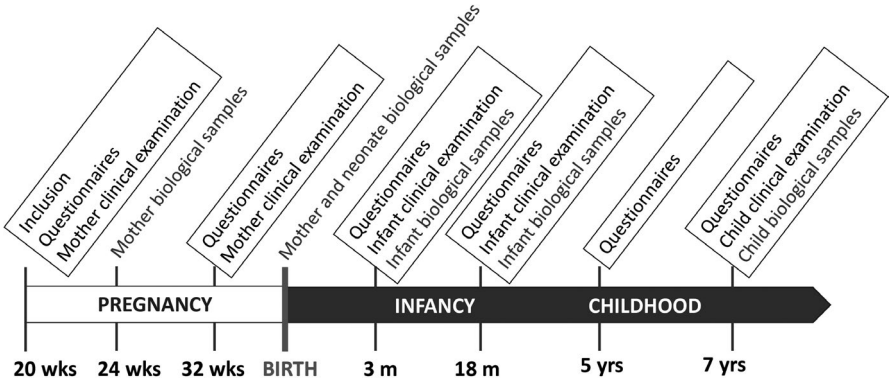

FIGURE 2 Summary of data collection flow in the NELA birth cohort study. Due to SARSCov-2 pandemic, only information by telephone is being collected at the follow-up visit of five years

TABLE 1 Summary of the information obtained by questionnaires and measurements from mothers at the different follow-up visits

	Pregnancy			Children's age			
	20–24 wk.	32 wk	Delivery	3 mo.	18 mo.	5 yr.	7 yr.
Information							
Obstetric & medical history	▲						
Asthma/allergy	▲						
Infection disease	▲	▲	▲				
Anxiety/depression	▲	▲		▲			
Tobacco smoking	▲	▲		▲	▲	▲	▲
Alcohol/drugs consumption	▲	▲		▲			
Diet & supplements	▲			▲			
Physical exercise	▲	▲					
Medicine intake		▲	▲				
Occupational hazards		▲					
Endocrine disruptors	▲		▲				
Outdoor air pollution	▲	▲	▲				
Measurements							
Weight	▲	▲	▲				
Height	▲						
Skin folds	▲	▲					
Body composition	▲	▲					
Blood pressure	▲	▲					
Lung function	▲						
Skin prick test				▲			
eVOCs[a]				▲			

[a] Exhaled volatile organic compounds.

administered by trained interviewers. The FFQ has semi-quantitative (112) and qualitative (11) items. For each food item, the questionnaire asks how often, of nine possible intake frequency categories, the participants have consumed a particular amount of food from the beginning of pregnancy until the time of the interview; and standard units or reference serving sizes are specified. The intake frequency for each food item was converted to the average daily intake. Then, nutrient values and energy intakes were obtained from different sources.[17,18]

To evaluate the degree of adherence to a Mediterranean Diet during pregnancy, two scores are being used: Alternative Mediterranean Diet score[19] (aMED) and Relative Mediterranean Diet score[20] (rMED) both a modified version of the Mediterranean Diet Score (MDS).[21] Other diet patterns will be also explored.

TABLE 2 Summary of the information obtained by questionnaires and measurements from children at the different follow-up visits

	Time point				
	Birth	3 mo.	18 mo.	5 yr.	7 yr.
Information					
Respiratory problems		▲	▲	▲	▲
Atopic eczema		▲	▲	▲	▲
Asthma/allergy		▲	▲	▲	▲
Skin prick test					▲
Cognitive development			▲		▲
Breast feeding		▲	▲		
Indoor tobacco smoking		▲	▲	▲	▲
Diet & supplements		▲	▲	▲	▲
Pet contact		▲	▲	▲	▲
Medicine intake		▲	▲	▲	▲
Vaccinations		▲	▲	▲	▲
Physical exercise			▲	▲	▲
Measurements					
Weight	▲	▲	▲	▲	▲
Height	▲	▲	▲	▲	▲
Head circumference	▲	▲	▲		▲
Waist circumference	▲	▲	▲		▲
Skin folds	▲	▲	▲		▲
Blood pressure					▲
Th1/Th2[a] & cytokines	▲				
Lung function		▲			▲
Outdoor air pollution	▲	▲	▲	▲	▲
eVOCs[b]		▲			

[a] T-helper (Th).

[b] Exhaled volatile organic compounds.

2.5.4 | Nutritional biomarkers

Maternal and offspring vitamin levels were quantified in maternal serum at 24 gestation week and in cord blood serum; 25-hydroxyvitamin D [25(OH)D] was measured by direct competitive immunoluminometric assay using coated magnetic microparticles in a LIAISON® XL automated analyser (DiaSorin S.p.A.). Both vitamin A and Vitamin E were extracted (CI&C GmbH) and quantified by HPLC (Waters, Barcelona, Spain). Fatty acids were analysed in maternal plasma at 24 gestation week in both arterial and venous cord blood to detect disturbances in their placental transfer and foetal fat uptake. They were quantified by gas chromatography on a Hewlett-Packard 6890 as previously described[22] (Agilent Technologies, Inc) equipped with a SP-2560 capillary column (60 m × 0.25 mm id × 0.15 μm; Supelco, SIGMA-Aldrich). Polyamines putrescine, spermidine and spermine were quantified by HPLC methods in both maternal serum at 24 gestation week and venous cord blood serum. Polyamines were derivatised to dansyl compounds and quantified using a reverse-phase column (Nova-Pak C18). A two-phase gradient, and detection by immunofluorescence was used.[23]

2.5.5 | Residential outdoor air pollution

To overcome the limitation of having too few air quality stations,[24] we followed the air quality Directive 2008/50/EC criteria for using high-resolution air quality models for complementing on-site observations. Thus, the Weather Research and Forecasting (WRF)+CHIMERE modelling system was developed for generating the outdoor concentrations for air pollutants during the entire duration of the NELA cohort study. To include the pregnancy period, simulations cover from nine months prior to the first enrolled women (July 2014) to the end of 2018.

The regional meteorological model Advanced Research Weather Research and Forecasting (WRF-ARW) Model v3.9.1.[25] was used to provide the meteorology to the chemistry transport model. WRF is driven every six hours by ERA-Interim reanalysis and has been coupled off-line on an hourly basis to CHIMERE chemistry transport model.[26] WRF fields are interpolated to CHIMERE working grids. MELCHIOR2 gas-phase mechanism is implemented within CHIMERE. The chemistry transport model includes gas-phase chemistry and aerosols; and heterogeneous chemistry distinguishes among different chemical aerosol components. The physico-chemical options for the regional modelling system are summarised elsewhere.[27]

With respect to anthropogenic and natural emissions, a specific emission inventory was developed for the purpose of the NELA study. Biogenic emissions are coupled to WRF outputs. The model estimates hourly isoprene, monoterpene and other biogenic VOC emissions.

The final working resolution over the three target Health Areas of NELA recruitment is 0.5 km. The exposure to air pollution is derived at short term (15 days before birth) and long term (entire pregnancy and trimester-specific). Information on residential address was collected in the follow-up visits. A geo-codification and geo-referencing process were implemented so the exposure to air pollution was extracted for the corresponding time-period and the exact geo-location of the mother-sib pair. This will be further applied at other time points of follow-up.

Exposure to indoor air pollution is being assessed through questionnaires in the different visits.

2.5.6 | Endocrine disruptors

Urine samples from the mothers were collected in the first visit. Samples were immediately frozen at −80°C. The main six organophosphate

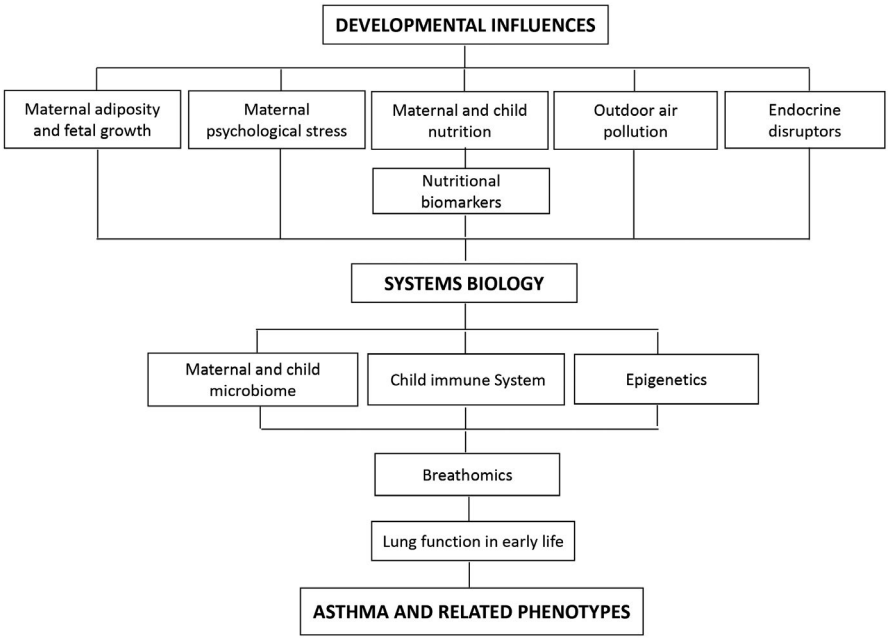

FIGURE 3 Diagram of the different research areas covered by the nutrition in early life and asthma (NELA) cohort and their interactions

pesticide metabolites (dialkylphosphates) such as dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyl-dithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) will be analysed in due course of time using gas chromatography coupled with tandem mass spectrometry (GC -MS/MS). Additionally, as markers of prenatal exposure to endocrine disruptors, anogenital distance (AGD) and penile width were measured at the birth visit, as previously described.[28]

2.5.7 | Psychological stress in the mother and child neurodevelopment

Psychological well-being during pregnancy was assessed by means of the Family Apgar test at 20 gestation week.[29] Additionally, the Edinburgh postnatal depression scale,[30] used to assess prenatal depression,[31] was administered at 20 and at 32 gestation week. Furthermore, the State-Trait Anxiety Inventory (STAI) test was included in the 32 gestation week visit.[32] Children were evaluated at 18 months of age by means of the Spanish version of the Bayley III scales for infant and toddler development.[33] During the same visit, externalising and internalising behaviour of parents was assessed using the Spanish version of the Child Behavior Checklist (CBCL) scale.[34] Their adaptive and social behaviour were also measured through the Behavior Assessment System-Third Edition (ABAS-III) scale.[35]

2.6 | Mechanisms: Systems biology

2.6.1 | Immune system phenotyping in the offspring

Measurements of immune system cells were performed by flow cytometry. Lymphoid cells were gated according to size (Forward Scatter, FSC) and granularity (Side Scatter, SSC) parameters. Frequency and absolute numbers of immune subpopulations Th1, Th2, Th17, Treg and ILC2 were obtained according to their cell surface markers.[36,37] Expression level of each marker was also analysed by using the mean fluorescence intensity data. Additionally, mononuclear cells from cord blood were isolated by density gradient centrifugation with Ficoll-Hypaque Plus (Amersham Biosciences).[38]

To obtain cord blood cytokine profiles, whole blood samples were incubated in the presence of specific immune stimulants or medium alone[39]:Concanavalin A, *D. pteronyssinus* and *Olea europaea* extracts, oligonucleotides containing CpG motifs, polyinosinic-polycytidylic acid (pI:C), peptidoglycan (PEG), lypopolisaccharide (LPS), and phytohaemaglutinin. Stimulants were chosen according to their capacity to induce innate and/or Th1, Th2 or Th17 cytokines in adults.[40] After incubation, supernatants were collected and analysed for the presence of the following cytokines: IFN-alpha, IFN-gamma, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17F, IL-23 and TNF-alpha. Concentrations of all these cytokines were measured simultaneously by the Luminex cytokine kit (Thermo Fisher Scientific) and run on a MAG-PIX (Luminex Corporation), equipped with xPONENT software (Luminex Corporation). Cytokine results were analysed by ProcartaPlex Analyst 1.0 Software (Thermo Fisher Scientific).

2.6.2 | Breathomics

Breath sampling, exhaled breath analysis and data preprocessing have been detailed recently.[41] Exhaled breath samples were collected from mothers and infants at three months of age in plastic free bags (Quintron™ and Tedlar™ gas bags for children, and Tedlar™ gas bags for mothers). Room air samples were also collected by an Easy-VOC syringe (Markes Int.™). The air contained in the sample bags was transferred to adsorption tubes for

trapping VOCs (Tenax TA, Markes Int.[TM]). The tube was sealed with brass caps fitted with PTFE ferrules and stored at 4 °C until analysis by thermal desorption coupled to chromatography-mass spectrometry (TD-GC/MS). Raw output data from GC/MS was preprocessed using a specifically created open code workflow in R language with functions from *xcms*, *cliqueMS* and *eRah*.[41] VOCs were identified through National Institute of Standard and Technology (NIST) spectral library matching by means of their retention time and their respective mass spectrum. In addition, chromatographic standards were used for retention indexes computation. Special attention will be paid to compounds previously related to asthma.[42,43]

2.6.3 | Maternal and child microbiome

Mothers attending during pregnancy to the Emergency Department of the hospital with any infection underwent sample extraction according to clinical suspicion. Additionally, two hundred mother-sib pairs were selected (according to their history of allergy) to obtain faecal samples (mother) or meconium (newborn). Furthermore, vaginal and naso-pharyngeal samples from the mother (COPAN eSwabs[TM], Copan Diagnostics, Italy) and an upper respiratory tract sample of the newborn (nasal lavage) were collected during delivery. All samples were cultured on different selective and non-selective media and incubated in aerobic and anaerobic conditions. Representative colonies of all morphotypes and sizes recovered were selected and purified by subculturing on the same medium and stored at −80°C until identification. Isolates will be identified by Vitek_MS (Biomerieux). Total DNA will be extracted. Quantification of the different bacterial populations will be carried out by quantitative PCR. All reactions are being performed in a CFX96 Real Time PCR System (Bio-Rad, Spain) using the SYBR[TM] Green PCR Master Mix (Thermo Fisher Scientific). Additionally, microbiota composition of the samples is being analysed using 16S rRNA amplicon sequencing with by Ion Torrent (Thermo Fisher Scientific). Previously, short-chain fatty acids were extracted from faecal samples.[44] Identifications and quantification are being performed by gas chromatography with a flame ionisation detector. Long-chain fatty acids were extracted by acid hydrolysis (ClH-methanol) and examined by GLC-MS Agilent system (GC 6890N, MS 5973N), working in Total Ion Chromatogram (TIC) and SIM-Selected Ion Monitoring (SIM) modes.

In addition, we have optimised a non-invasive method for transcriptome analyses of exfoliated human intestinal cells isolated from faecal samples.[45]

2.6.4 | Epigenetics

Cord blood DNA methylation has been measured at 866,836 CpG sites on the EPIC BeadChip (Illumina). Quality control (QC) measures included use of repeats and randomisation of samples across chips and plates, and both sample and probe filtering.[46] Background signal has been subtracted and corrected for dye bias using "noob" (normal-exponential convolution using out-of-band probes) and normalised for differences between type I and type II probes using beta mixture quantile normalisation (BMIQ).[47,48] Moreover, leucocyte proportions have been estimated using CP-QP (constrained projection quadratic programming) applying a reference for cord blood.[49,50]

In addition, venous cord blood samples collected using PaxGene tubes (Quiagen, Valencia, CA) were processed to extract RNA enriched with miRNAs. The microarray technology by Agilent was used to analyse the expression profile at birth of 2549 miRNAs by SurePrint G3 Human miRNA kit v.21.

2.7 | Sample size and statistics

To detect a clinical significant change in lung function (half a SD) as measured by forced vital capacity at 0.5 s (FEV 0.5) in the infants exposed to a healthy diet as compared with an unhealthy one (upper vs. lowest tertiles of diet score) and 50% dropouts, the sample size should be 110 individuals allowing for a mean FEV0.5 of 70 ml and a SD of 15 ml (9), together with a significant level of 0.05 and a power of 0.8. To have at least a double number to maintain robustness in multiple comparisons, 220 individuals are advised in each tertile of diet score, that is, a total of 660, including dropouts. Our aimed 800 mother-child pairs should be quite enough to detect any significant effect in FEV0.5. Additionally, 800 individuals would suffice for multivariate analyses with a dichotomous dependent variable (wheezing vs. non-wheezing, for instance) including many potentially predictive ones (up to 80 as a rule of thumb). Participation rate is anticipated to be much higher in procedures other than lung function testing, where the infant should be sedated. In this regard and according to previous data from the region,[51] at least 300 out of 800 infants will have one or more wheezing episodes during the first year of life, which will allow for enough power provided the obtained lung function tests are not enough due to rejection by parents. The balance between cases and controls (300 vs. 500) would allow to detect a difference in the proportion of methylation of 7% with a significance level of 10−6 and a power >80%. Apart from the usual statistical methods for uni- and multivariate analyses, mediation analyses will be considered when appropriate. Analyses will be performed with Stata V15-17 software (College Station, TX, USA) and R. No imputation of lost data will be attempted.

2.8 | Ethics approval

All procedures included in the cohort study were approved by the ethics committee of the "Virgen de la Arrixaca" University Clinical Hospital (report 9/14; 29/09/2014).

TABLE 3 Participation rate[a] in the Nutrition in Early Life and Asthma (NELA) cohort study up to the 18 months visit of the children

	32 weeks	Birth	3 months	18 months
Visited	721	720	612	532
Not visited	17	18	108	188
Withdrawals	11	16	13	27
Loss of follow-up	5	0	95	161
Miscarriages	1	2	-	-
Participation rate (%)	97.7	97.6	82.9	72.1

[a] A total of 738 mother-child pairs agreed to participate at week 20 of gestation, after inviting 1350 over a period of 3 years (54.7% participation rate in the recruitment period).

TABLE 4 Baseline characteristics of mother-child pairs included in the NELA birth cohort study

Characteristics	N[a]	N (%) or mean ± SD
Maternal age, years	738	32.6 (4.7)
Maternal education	738	
Primary or less (8 years or less)		57 (7.7)
Incomplete secondary (9–11 years)		89 (12.1)
Complete secondary (12 or more)		191 (25.9)
University		401 (54.3)
Parity, primiparous	738	374 (50.7)
Maternal smoking in pregnancy		
During first trimester	738	124 (16.8)
During second trimester	738	109 (14.8)
During third trimester	674	88 (13.1)
Maternal asthma	738	81 (11.0)
Maternal allergic rhinitis	738	203 (27.5)
Maternal atopic dermatitis	738	67 (9.1)
Maternal atopy	738	310 (42.0)
Paternal age, years	738	34.8 (±5.3)
Paternal education	736	
Primary or less (8 years or less)		107 (14.5)
Incomplete secondary (9–11 years)		111 (15.1)
Complete secondary (12 or more)		229 (31.1)
University		289 (39.3)
Paternal smoking	736	258 (35.1)
Paternal asthma	736	66 (9.0)
Paternal allergic rhinitis	736	175 (23.8)
Paternal atopic dermatitis	736	37 (5.0)
Paternal atopy	736	263 (35.7)
Newborn sex (male)	720	357 (49.6)
Gestational age at birth (weeks)	720	39.6 (± 1.5)
Preterm birth (<37 weeks of gestation)	720	36 (5.0)
Birthweight (g)	712	3242 (± 475)
Low birthweight (<2500 g)	712	42 (5.9)
Caesarean section	712	157 (22.1)

[a] Total number of individuals in which the information is available

3 | RESULTS

3.1 | Recruitment and participation

NELA started in March 2015 and the recruitment period extended into 2018. Among the 1350 women invited to participate, 738 (54%) were finally enrolled in the study. Reasons for non-participation were: 37% did not want to participate, 33% said that they did not have time, 2% reported no interest and 28% did not offer any explanation. Excluding 16 withdrawn, one miscarriage and one stillbirth, there were 720 eligible mother-offspring pairs at birth. Participation rates at the different follow-up visits are shown in Table 3.

3.2 | Demography

On average, women were included at 23 weeks of gestation (range 19, 22). The baseline characteristics of the participants are shown in Table 4. Asthma was diagnosed to 11% of mothers, which compares quite well with the prevalence among fathers (9%) and with the general population in Spain which had increased from 4.5% in 1997 to 7.3% in 2007,[52] with expected increase in the last decade. The prevalence of preterm births was 5%, also quite comparable with 6.7% found in a big survey in Spain carried out in 2015.[53]

3.3 | Lung function tests

The number of infants whose lung function was measured was disappointingly low as compared with the expectations. Although numerous attempts to improve participation were implemented, including the procedure and its harmlessness being extensively explained to parents by the two paediatricians included in the study, parents were very reluctant to their children being sedated with chloride hydrate. The total number with valid data in the lung function data set was 76 infants, with a mean age at measurement of 21.2 ± 4.4 weeks.

3.4 | Nutritional data

Diet was surveyed at the visits depicted in Table 1 (mothers) and 2 (children). The number of food questionnaires from mothers were,

TABLE 5 Biological samples collected so far in the NELA birth cohort study

Biological sample	Time point			
	24 weeks of pregnancy	Delivery	3 months of child's age	18 months of child's age
Mothers				
Venous blood				
Plasma	710	418		
Serum	711	416		
White blood cells[a]	680	413		
Lymphocytes	698	379		
RNA	697	419		
Swabs				
Vaginal		409		
Nasal		427		
Pharyngeal		424		
Rectal		407		
Placental tissue		404		
Urine	683	399		
Hair	662			
Breast milk			240	
Exhaled air			337	
Children				
Cord blood vein				
Plasma		390		
Serum		380		
White blood cells[a]		390		
Lymphocytes		341		
RNA		384		
Cord blood artery		179		
Plasma[b]		179		
White blood cells		249		
Lymphocytes[c]		12		
Swabs				
Vermix		407		
Nasal		407	609	507
Meconium/faeces		364	538	464
Urine			511	266
Hair			503	507
Exhaled air			354	

[a] DNA currently being extracted from white blood cells.

[b] In numerous samples, plasma was haemolysed and could not be stored.

[c] Due to the difficulty in extracting arterial cord blood, two different tubes were only obtained in 12 new-borns. White blood cells were prioritised.

respectively, 665 (90.1%) and 574 (93.8%) for 20th gestation week and for the three months of children's age. The number of questionnaires obtained from mothers at 18 months of age of their children was 532 (100%).

3.5 | The NELA biobank

Samples obtained in the study are stored in the BioBank "Biobanco en Red de la Región de Murcia" (PT17/0015/0038 & PT20/00109),

TABLE 6 Birth cohort studies with the potential of merging data with the NELA cohort at some follow-up point

Cohort name	Year started	Starting N	Population	Infant lung function tests	Prenatal information (any)
Tucson Children's Respiratory Study (TCRS)	1980	1246	General	YES (RTC[a])	NO
Childhood Asthma Study (CAS-I and CAS-II)	1987	835	General	NO	NO
Perth Infant Asthma Follow-up (PIAF)	1987	254	General	YES (RTC[a])	NO
The Isle of Wight Birth Cohort	1989	1456	General	NO	NO
Multicentre Allergy Study (Multizentrische Allergiestudie) (MAS)	1990	1314	General enriched with high risk	NO	NO
Avon Longitudinal Study of Parents and Children (ALSPAC)	1991	14,541	General	NO	YES
Environment and Childhood Asthma study (ECA)	1992	3754	General	NO	NO
Oslo Research Group for Asthma and Allergy in Childhood, the Lung and Environment (ORAACLE)	1992	3754	General	YES (tPTEF/tE[b])	NO
Stockholm Children Allergy and Environmental Prospective Birth Cohort Study (BAMSE)	1994	4089	General	NO	YES
Canadian Asthma Primary Prevention Study (CAPPS)	1994	545	High risk	NO	YES
Epidemiology of Home Allergens and Asthma Study (EHAAS)	1994	544	High risk	NO	YES
German Infant Nutritional Intervention plus environmental and genetic influences on allergy development (GINIplus)	1995	5991	General (control) High risk (intervention)	NO	NO
Manchester Asthma and Allergy Study (MAAS)	1996	1184	General	NO	YES
Prevention and Incidence of Asthma and Mite Allergy (PIAMA-NHS)	1996	3963	General	NO	YES
AMICS-INMA Menorca	1997	482	General	NO	YES
Columbia Center for Children's Environmental Health (CCCEH)	1997	727	Low-income minority	NO	YES
Infant Immune Study	1997	482	General	NO	YES
Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus Air Pollution and Genetics (LISAplus)	1997	3097	General	NO	YES
Childhood Origins of ASThma (COAST)	1998	287	High risk	NO	NO
Southampton Women's Survey (SWS)	1999	2567	General	YES (RVRTC[c])	YES
The Copenhagen Study on Asthma in Childhood 2000 (COPSAC2000)	1998	411	High risk	YES (RVRTC[c])	YES
The Norwegian Mother and Child Cohort Study (MoBa)	1999	109000	General	NO	YES
Project Viva	1999	2128	General	NO	YES
Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS)	2001	762	High risk	NO	NO
The Wheezing Illnesses Study Leidsche Rijn (WHISTLER)	2001	2150	General	YES (Crs, Rrs, Trs[d])	YES
Etude sur les Déterminants pré et post natals précoces du développement psychomoteur et de la santé de l'Enfan (EDEN)	2003	2002	General	NO	YES
Rome and Bologna Birth Italian Cohorts (ROBBIC)	2003	1369	General	NO	NO
Wayne County Health, Environment, Allergy & Asthma Longitudinal Study (WHEALS)	2003	1258	General	NO	YES

(Continues)

TABLE 6 (Continued)

Cohort name	Year started	Starting N	Population	Infant lung function tests	Prenatal information (any)
INfancia y Medio Ambiente Project [Environment and Childhood] (INMA)	2004	2021	General	NO	YES
ECUADOR-Life (ECUAVIDA)	2005	2404	General	NO	NO
Urban Environment and Childhood Asthma (URECA)	2005	609	High risk	NO	YES
Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE)	2006	1484	General	NO	YES
COhort for Childhood Origin of Asthma and allergic diseases (COCOA)	2007	2500	General	NO	YES
Mother-Child cohort in Crete (Rhea)	2007	1479	General	NO	NO
The Canadian Healthy Infant Longitudinal Development Study (CHILD)	2008	3523	General	NO	YES
Vitamin D Antenatal Asthma Reduction Trial (VDAART)	2009	890	High risk	NO	YES
The Copenhagen Studies on Asthma in Childhood (COPSAC2010)	2010	700	General	NO	YES
Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé (SEPAGES)	2014	484	General	YES (tPTEF/tE[b])	YES
Early origins of allergy and asthma (ARIES)	2020	250	General	NO	YES

Note: All cohorts included have information on asthma diagnosis, symptoms, and lung function tests (spirometry) at school age. When indicated, the cohort has information (with the method indicated) about infant lung function (usually only in a small portion of them), and about prenatal risk factors (any). The information included comes from https://asthmabirthcohorts.niaid.nih.gov/ and from recent literature search. It is possible that some cohorts have been missed in spite the thorough search.

[a] Rapid Thoraco-abdominal Compression.

[b] Time taken to achieve peak tidal expiratory flow as a proportion of total expiratory time.

[c] This is not a proper asthma cohort, but a limited number of mothers were approached to perform lung function tests in their children. A total of 362 mothers were approached and 131 accepted.

[d] Raised Volume Rapid Thoraco-abdominal compression.

[e] Total thoracic compliance (Crs), resistance (Rrs) and respiratory system time constant (Trs).

integrated in the Spanish National Biobanks Network (B.000859) after being processed following established protocols. The number, types and origin of the biological collection are included in Table 5.

3.6 | Potential for collaboration

The NELA cohort can collaborate with existing or future population-based asthma-specific asthma cohorts. Most cohorts shown in Table 6 can share information on exposures and outcomes with NELA; however, the most interesting ones are those which include very early lung function testing, such as TCRS, PIAF, COAST, SWS, COPSAC2000 and SEPAGES. Of them, the two that have the same method for measuring lung function are COPSAC2000 and SWS (Table 6).

4 | COMMENT

Implementing a mother-child cohort focused on maternal diet during pregnancy is complex and requires the participation and coordination of many researchers and clinicians with specific expertise in many disciplines. The NELA cohort offers valuable information and biological samples which could add some light to the origins of asthma and other NCDs. Therefore, it might contribute to their primary prevention, reducing their burden, both through increasing the population health and through reducing the suffering and the economic costs.

4.1 | Principal findings

Some early findings have been already published and are available in four scientific articles. The first one shows that traffic-related air pollution impairs foetal immune balance affecting the numbers of cytotoxic T lymphocytes and NK, Th1 and Treg cells from cord blood.[54] The second one displays an open-source workflow for preprocessing the raw data from exhaled breath analysis.[41] The third one shows that maternal docosahexaenoic acid (DHA) supplementation during pregnancy increases foetal DHA regardless of the pre-pregnancy BMI, while gestational diabetes mellitus may reduce the

effect of DHA supplementation in newborns.[55] The last one found that younger mothers and those with previous deliveries have a greater probability of low adherence to the Mediterranean diet.[56]

4.2 | Strengths of the study

The NELA cohort is somewhat unique as, to the best of our knowledge, it is the first one implementing the measurement of eVOCs by GC/MS in exhaled breath from infants as potential early markers of respiratory disease[43]; and it is also one of the three which measured lung function in the early infancy by means of the RVRTC technique[57,58] (Table 6).

The cooperation of basic researchers, clinicians and epidemiologists needed to implement the study will be easily maintained. This would allow integrating all possible information obtained from very different approaches, permitting a more extensive understanding of the early origins of asthma and other NCDs.

The number, diversity and aliquots of biological samples obtained so far, and those that will be obtained in the future, offer a considerable potential to study and find the mechanistic pathways to explain causal associations between risk factors and outcomes.

Although the participation rate of pregnant women invited to join was relatively low, once they entered the study, the rate in the subsequent visits from week 20 of gestation until month 18 of children has been quite high. Many included mothers were in their first pregnancy, although the mean age was not young, reflecting the current trend in many countries of women having their first child in their thirties.

Furthermore, according to the prevalence of asthma in mothers and fathers, and that of preterm births, the population in the NELA cohort is probably a good representation sample of the whole population in the area.

4.3 | Limitations of the data

The main limitation of the NELA cohort is the relatively low number of participants (still comparable to most other cohort studies) which might not offer enough power to detect weak associations between exposures and outcomes, especially in multivariable analyses. Furthermore, the number of biological samples in certain cases is even lower, further endangering the power of statistical analyses. The low participation rate is of special importance in the lung function tests, in which only 76 usable ones were obtained. Being healthy infants, and although many efforts were made, it was very difficult to convince parents to have their children sedated. However, the number of infants with lung functions test is considerable when the present series is compared with others in the literature.[59] Those tests may still allow for certain analyses, such as the association between VOCs and lung function stated previously, and others. Hopefully the data from the present cohort could be combined with that of other similar cohorts to obtain enough statistical power.

4.4 | Interpretation

The NELA cohort is a representative sample of the general population in which risk and protective factors of asthma and other NCD can be studied. The follow-up has been reasonably successful so far with participation rates comparable to similar cohorts, which will allow analysing at least 532 infants 18 months of age and hopefully a similar number at 5 years, which is the visit currently ongoing. Unfortunately, the COVID-19 pandemic has forced it to be telephonic, but we expect to be able to have another visit at 7 years of age in which spirometry can be performed with no difficulty.

5 | CONCLUSIONS

The NELA cohort will allow adding original and unique information to the developmental origins and mechanisms of asthma. It can also identify new biomarkers of asthma risk and prediction in exhaled breath at very early ages; and has the potential of unravelling some of the pathways which might cause asthma. Additionally, the cohort could add information on the early life origins of other NCDs.

To conclude, the cohort will also offer an enormous amount of data from questionnaires, physical examinations and measurements at different ages, together with analyses of biological samples either performed or being performed in the future through novel technologies, using biobanked samples. This present and upcoming information will be offered to contemporary and future researchers to try and better understand the origins and mechanisms of asthma and other chronic conditions under the light of the DOHaD paradigm.

ACKNOWLEDGMENTS

We are extremely grateful to all the families who participate in this study, the hospital technicians, midwifes and gynecologists for recruitment and following them up, and the whole NELA team for their commitment and their role in the success of the study. We want to particularly acknowledge the BioBank "Biobanco en Red de la Región de Murcia" (PT17/0015/0038 & PT20/00109) integrated in the Spanish National Biobanks Network (B.000859) for its collaboration in creating and maintaining the NELA biobank.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

The study protocol including all questionnaires and measurements of all visits already performed are available in the NELA website (https://nela.imib.es/) upon request. The NELA data, including de-identified individual participant data, will be made available to interested researchers by the NELA Steering Committee. Access will require a formal request, a written proposal and a signed data access agreement.

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How to cite this article: Morales E, Alcantara-Lopez MV, Cabezas-Herrera J, et al; the NELA Study Group. The Nutrition in Early Life and Asthma (NELA) birth cohort study: Rationale, design, and methods. *Paediatr Perinat Epidemiol*. 2021;00:1–15. https://doi.org/10.1111/ppe.12826