POLISH MOTHER AND CHILD COHORT STUDY (REPRO_PL) – METHODOLOGY OF THE FOLLOW-UP OF THE CHILDREN AT THE AGE OF 7

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Abstract
Effects of environmental exposures in utero and in the first years of life on early life health and development is a growing research area with major public health implications. The main aim of this work has been to provide an overview of the next step of the Polish Mother and Child Cohort Study (REPRO_PL) covering exposure, health and neurodevelopment assessments of children at 7 years of age. Details regarding methodology of the follow-up of the children are crucial for cross-cohort collaboration and a full understanding of the future research questions. Phase III of the REPRO_PL cohort covers a follow-up of 900 children at the age of 7 years old. The questionnaire filled in by the mothers is composed of: socio-demographic, child exposure and home environment information, nutritional status and health data. In the case of 400 children, environmental (including collection of urine, saliva and buccal cells), health status and psychomotor assessments are performed. Health and development check consists of physical measurements, child health status assessment (including lung function tests, skin prick testing, an interview/examination by an allergist) and psychomotor development tests (the Strength and Difficulties Questionnaire and the Intelligence and Development Scales). The results of the study will become available within the next few years. Extension of the REPRO_PL cohort with examinations of children at the age of 7 years old may provide a better understanding of the relationship between environmental and lifestyle-related factors and children’s health and neurodevelopment; and may further strengthen scientific base for policies and interventions promoting healthy lifestyle. Int J Occup Med Environ Health 2016;29(6):883–893

Key words:
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INTRODUCTION
Exposure to environmental and lifestyle factors during prenatal, or even preconceptual period, and in the first years of life may have an impact on the course and outcome of pregnancy as well as on a child’s development and health across the whole life course [1,2]. Birth cohort studies are ideally suited to improve causal inference in this field [3]. They are designed to study the impact of early exposures prospectively and at multiple time points during development of a child [3]. Furthermore, birth cohort studies collect biological samples from mothers and children, enabling measurements of biomarkers of exposure, its effects, or susceptibility [3].

Over the past 25 years, more than 50 pregnancy and birth cohorts have been established in 19 European countries with 500 000 life born children in total. This provides a unique opportunity to examine the associations between early-life exposure and child health and development [3,4]. Considering the fact that cohorts are expensive to maintain and might not have sufficient power for some associations, the value of a cross-cohort collaboration with pooling data from different cohorts needs to be strengthened. In addition, such a collaboration allows cross-cohort comparisons. Detailed information on characteristics of pregnancy and birth cohorts might also be essential for investigators of new cohorts, who might benefit from such resources [4].

Polish Mother and Child Cohort Study (REPRO_PL) is a multicenter prospective cohort study established in 2007 with the aim to evaluate a variety of environmental factors contributing to the pregnancy outcomes, children’s health and neurodevelopment. The details regarding exposure (including biological samples collection) and outcome variables focusing on pregnancy period and the first 2 years of life have been presented on the following websites: http://www.repropl.com (website dedicated to REPRO_PL cohort [5]), http://www.enrieco.dk, http://www.birthcohorts.net (websites dedicated to existing mother-child cohorts [6,7]), in 2 publications focused on REPRO_PL [8,9] and 2 concerning European birth cohorts [3,4]. In addition to the analyses performed only within the REPRO_PL sample, the data from this study was also included in combined analyses from a few European cohorts [10–12]. To utilize the already performed work it is crucial to follow up the children to address new hypotheses or to evaluate whether the observed associations still persist in the school age.

The main aim of this work has been to provide an overview of the next step of the REPRO_PL study covering the exposure and health as well as the assessment of neurodevelopment among 7-year-old children.

The details regarding methodology of the follow-up of the children are crucial for a cross-cohort collaboration and a full understanding of the future research questions.

OBJECTIVES
REPRO_PL aims at evaluation of the impact of exposure to environmental and lifestyle-related factors during pregnancy and after birth on pregnancy outcomes and children’s health.

As regards the children’s health and development within the 7 years of life, the study’s 3 specific aims refer to:

– assessment of the impact of prenatal exposure to maternal lifestyle-related factors (tobacco constituents, alcohol consumption, pre-pregnancy body mass index (BMI), physical activity, stress, phthalates and lead exposure, folic acid intake) as well as the exposures within the first 7 years of life (environmental tobacco smoke (ETS) and phthalates) on a child’s neurodevelopment at the age of 7;

– assessment of the impact of prenatal exposure to maternal lifestyle-related factors (tobacco constituents, phthalates, trace elements, vitamin D) and the exposures within the first 7 years of life (ETS and phthalates exposure) on a child’s allergy and asthma at the age of 7;

– assessment of the impact of maternal pre-pregnancy BMI, weight gain in different periods of pregnancy
and early child’s psychomotor development as well as young children overweight/obesity on the older children’s risk of a low physical activity and adiposity outcomes.

The list of research objectives is not final as additional questions may be formed and answered along with obtaining results of the REPRO_PL study.

Information about prenatal and early postnatal exposure needed for some of the above aims has been already collected and analyzed (a detailed description of the methodology related to the pregnancy period was published in previous papers) [8,9]. This article describes the methodology for the follow-up of children from REPRO_PL cohort at the age of 7 years old.

Current stage of the study

Details regarding Phase I of the REPRO_PL study covering pregnancy period have been published elsewhere [8]. The Figure 1 shows the structure and response/follow-up rate of the REPRO_PL cohort. We obtained all the scheduled data during pregnancy and after the delivery as well as detailed information on the newborns from 1045 women out of 1763 women who fulfilled the inclusion criteria and agreed to participate in the study (59.3%). At least one visit during pregnancy was made and data on the newborn was obtained in the case of 221 women (12.5%), and in the case of 368 women (20.9%) at least 2 visits during pregnancy took place but the outcome data was not available. The remaining mothers either experienced a miscarriage/infant death (42 women, 2.4%), refused to participate in the follow-up visits (82 women, 4.7%) or were lost in the follow-up (5 women, 0.3%).

Taking into account organizational feasibility of the study, Phase II of the REPRO_PL cohort covering child examinations at the age of 1 and 2 was realized in 2 regions of Poland (Łódź and Legnica) (details are published elsewhere) [9]. At that stage of the study, we examined 547 out of 876 children who were invited to participate (62.4%). Among them, 310 children had both examinations (at the age of 1 and 2), 199 were examined at the age of 1 year and 38 only at 2 years of age (Figure 1). Reduction in the follow-up numbers resulted from withdrawal, child health problems and loss-to-follow-up due to an unknown address and telephone number.

What has been found based on REPRO_PL?

A full list of publications is available on the REPRO_PL website (http://www.repropl.com [5]). A number of results based on the REPRO_PL data have indicated that maternal lifestyle during pregnancy and child environment after birth have a significant impact on child’s health and psychomotor development within the first 2 years of life. Children prenatally exposed to: a) tobacco compounds, b) lead, c) maternal stress, d) phthalates, as well as those of underweight mothers had decreased psychomotor development [13–15]. The recommended level of leisure-time physical activity (LTPA) during pregnancy had a positive impact on child development [15].

The results of REPRO_PL have also indicated that higher zinc and copper concentrations in cord blood were associated with the increased likelihood of wheezing in 1-year-old children [16,17]. This effect was seen only among the children exposed to tobacco smoke at home. In addition, a significantly lower activity of glutathione peroxidase enzyme in cord blood plasma of the children with atopic dermatitis during the first year of life was observed [16,17]. The cord blood 25-hydroxyvitamin D (25(OH)D) levels were inversely associated with the risk of multi-triggered wheezing and, especially, viral-induced wheezing, whereas phthalate exposure increased the risk of food allergy by the age of 2 years old [18,19].

It is crucial to evaluate whether the above associations still persist in the early school age or are compensated by other factors. A better understanding of the relationship
* Scheduled visits: a) during pregnancy: visit A (8–12th week of pregnancy), visit B (20–24th week of pregnancy), visit C (30–34th week of pregnancy), b) within 1 week after delivery (visit D).

**Fig. 1.** Structure of the Polish Mother and Child Cohort (REPRO_PL) – Phase I: pregnancy period, Phase II: child examination at 1 and 2 years of age, and Phase III: child examination at 7 years of age
between environmental and lifestyle-related factors and children’s health and neurodevelopment may further strengthen scientific base for policies and interventions promoting healthy lifestyle.

Follow-up of the children at the age of 7
(Phase III of REPRO_PL)
To confirm whether associations observed previously within the REPRO_PL study still persist in children in the early school age, and to achieve the 3 objectives of this phase of the study, the follow-up of about 900 children has been continued up to the age of 7 years old (considering the expected response rate at the level of 55% from 1633 mother–child pairs who participated in Phase I, 900 pairs will be followed-up up to the age of 7 years old). Taking into account organizational feasibility, the full assessment will be performed among 400 children from Łódź and Legnica regions (Group 1). Additionally, detailed questionnaires will be sent to mothers of 7-year-old children in the number of 500 from REPRO_PL from other regions with a request to be filled in by a mother of each child (Group 2) (Figure 1 and Table 1).

Questionnaires for exposure, health status and development assessment
The questionnaire composed of several parts will be filled in by mothers of the children participating in the study. The first part of the questionnaire covers basic socio-demographic information, child exposure and home environment, i.e., house size, pets at home, any molds and damps visible at home and exposure to ETS (covering source and duration of exposure). Additionally, the questionnaire provides information on the child’s physical activity (type of activity, time spent on the activity, information about the time spent on watching TV and playing computer games) and school curriculum.
A separate part of the questionnaire focuses on the children’s health including: severity and frequency of diseases, hospitalizations, medications taken and information about doctor-diagnosed allergy and asthma [20].
Apart from the basic questionnaire, mothers will also fill in a 24-h dietary recall questionnaire (24HR) and the Eating Maturity Questionnaire. Within 24HR, mothers are asked to report all food and beverages consumed by their children in the preceding 24 h [21,22]. The questionnaires are filled in for 3 days (two weekdays and one weekend day) and are supported by an “Album of photographs of food products and dishes” [23]. Based on such data, details regarding calories and nutrients obtained with the consumed food are calculated. The Eating Maturity Questionnaire, a self-reported 21-item tool consisting of 2 subscales: Rational Eating and Psychosocial Maturity, measures eating maturity that initiates and gives direction to human eating behaviors [22].
Finally, a mother of each child will fill in in the Parental Attitudes Scale [24]. The test contains 50 diagnostic statements, grouped in 5 dimensions, that correspond to 5 parental attitudes: acceptance-rejection, excessively demanding, autonomy, inconsequent, excessively protecting.

Assessment of exposure
Details regarding exposure to environmental and lifestyle related factors during prenatal and early postnatal period are already available based on the questionnaire data or biomarkers measurements (Table 1). Data on the exposure status at the age of 7 years old will be obtained from the questionnaire filled in by mothers of 900 children involved in the study as described above. In addition, urine, saliva and buccal cells will be collected from all the children. All the samples will be then stored at ~80°C and will be available for further analyses. This allows to assess the exposure and individual susceptibility. The analyses of phthalate, polycyclic aromatic hydrocarbons and environmental tobacco smoke exposure and oxidative stress biomarkers are intended as the continuation of those performed within Phase I and II of the study.
| Variable | Data source | Biological sample collected | Health status measurements |
|----------|-------------|-----------------------------|---------------------------|
| Parents  | questionnaire data | biological sample collected | health status measurements |
| socio-demographic variables | family structure, financial situation, parental age and education (during pregnancy and within 7 years after a child birth) | samples collected from the mothers during pregnancy and at delivery (saliva, blood, urine, hair, cord blood); assessments available for some exposures: cotinine, phthalate, PAH, PM\textsubscript{10}, microelements, vitamins, Pb, Cd, Hg | |
| lifestyle and nutrition | maternal lifestyle during pregnancy based on the questionnaire (pre-pregnancy BMI, weight gain in pregnancy, FFQ, physical activity, smoking, ETS, alcohol), parental lifestyle within 7 years after a child birth | | |
| psychosocial conditions | maternal stress during pregnancy (Subjective Work Characteristics Questionnaire, Perceived Stress Scale and Social Readjustment Rating Scale), Parental Attitudes Scale and Eating Maturity Questionnaire (in the case of children at the age of 7 years old) | | |
| health status of the parents | parental history of allergy and asthma diseases, paternal anthropometric measurements (in the case of children at the age of 1, 2 and 7 years of age) | | maternal health status and anthropometric measurements during pregnancy |
| Children | lifestyle, nutrition, health | home environment, ETS exposure, physical activity (at 1, 2 and 7 years of age), school curriculum, 24-h dietary recall questionnaire (at 7 years of age) | urine (at 1, 2 and 7 years of age), saliva, and buccal cells (at 7 years of age) | birth outcomes (gestational age, birth weight, length, head and chest circumference), physical measurements: height and weight (at 1, 2 and 7 years of age), waist and hip circumference, body composition measures, blood pressure (at 7 years of age), child health status assessment (after birth, at 1, 2 and 7 years of age), lung function tests, skin prick testing (at 7 years of age) Bayley Scales of Infant and Toddler Development (at 1 and 2 years of age) Intelligence and Development Scales (at 7 years of age) |
| | neurodevelopment | Strength and Difficulties Questionnaire (at 7 years of age) | | |

BMI – body mass index; FFQ – food frequency questionnaire; ETS – environmental tobacco smoke; PAH – polycyclic aromatic hydrocarbons; PM\textsubscript{10} – particulate matter with an aerodynamic diameter of $\leq 10$ μm; Pb – lead; Cd – cadmium; Hg – mercury.
Child health and psychomotor development assessment at the age of 7

We will assess health status and psychomotor skills among 400 children living within Łódź and Legnica regions. Health and development check consists of physical measurements, child health status assessment and neurodevelopment test battery. Physical measurements include anthropometric measurements (height, weight, waist and hip circumference), body composition measures (fat mass and fat-free mass) by means of the bioelectrical impedance analysis and blood pressure. Child weight and height are measured using standard protocols, without shoes and in light clothing. Waist circumference is measured in a standing position at the midpoint between the lowest rib margin and the iliac crest after a gentle expiration. Systolic and diastolic blood pressure is measured by the specially trained personnel of the research team twice after at least 5 min in a resting position.

A child examination by a pediatrician/allergist is accompanied by an interview with mothers concerning child health status in the previous years. Among the kids, the following tests are performed: pulmonary function testing, the exhaled nitric oxide measurements and skin prick testing. Pulmonary function testing is conducted using a MasterScreen unit (Erich Jaeger GmbH, Höchberg, Germany). Flow-volume curves are performed according to the American Thoracic Society standards [25]. The highest of 3 successful measurements is recorded and expressed as percentages of the predicted values. Reversibility testing is performed after administration of salbutamol (200 μg). The percentage change from the baseline in forced expiratory volume in 1 s (FEV₁), pre-bronchodilatory FEV₁ and peak expiratory flow (PEF) are presented.

The exhaled nitric oxide measurements are performed according to the European Respiratory Society/American Thoracic Society (ERS/ATS) recommendations by the use of a chemiluminescence analyzer (model 280i nitric oxide analyzer; Sievers, Boulder, CO, USA). The subjects exhale at a constant flow rate (50 ml/s) from total lung capacity to residual volume without breath holding. They maintain a constant mouth pressure (17 cm H₂O) by monitoring a visual display in order to eliminate contamination from nasal nitric oxide (NO). The mean value of 3 successive, reproducible recordings will be retained for a statistical analysis.

Skin prick testing (SPT) is performed with the most common inhalant allergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria*, *Cladosporium*, cat dander, dog dander, mixed grass pollen, rye, birch, hazel, ribwort, alder, mugwort together with a positive (histamine chloride 10 mg/ml) and negative (glycerol) control (extracts from Allergopharma-Nexter, Reinbeg, Germany). A positive SPT reaction is defined as a mean weal diameter > 3 mm in excess of the negative control.

The assessment of child’s psychomotor development includes the Strength and Difficulties Questionnaire (SDQ) filled in by mothers, and the evaluation of a child conducted by a certificated psychologists by means of the Intelligence and Development Scales (IDS). The SDQ (parent version) is a validated and widely used screening instrument to detect mental health difficulties in children and adolescents. The test consists of 5 subscales: “emotional problems,” “conduct problems,” “hyperactivity/inattention,” “peer problems,” and “prosocial behavior.” The cutoffs of the Polish validation study will be used to classify children as having “unlikely,” “possible,” or “probable” difficulties in the SDQ domains and the SDQ total scale. Internal reliability of the whole scale is satisfactory (Cronbach’s α = 0.8) [26]. The IDS has been adapted to Polish population by Jaworowska et al. [27]. The test is performed by psychologists and consists of 19 subtests representing 6 domains: Cognitive abilities, Psychomotorics, Language, Logical-mathematic thinking, Achievement motivation, Socio-emotional competencies. Thirteen subtests concerning 4 domains (excluding Socio-emotional competencies and Achievement motivation) will be employed in...
our research. Thus, we will identify fluid intelligence, crystallized intelligence and psychomotor abilities. In the case of 500 children from regions other than Łódź and Legnica, it will be possible to obtain child health and development information from the questionnaires filled in by their mothers. They will cover the SDQ, height and weight information, severity and frequency of diseases, any hospitalizations, medications taken and information about doctor-diagnosed allergy, and asthma.

**Strengths and weaknesses of the REPRO_PL cohort study**

The study’s main strength relates to its unique design – a prospective birth cohort. The study was implemented in early pregnancy with several follow up visits throughout the pregnancy period, and a detailed follow up of the children’s health and development at the age of 1, 2 and 7 years old. By restricting our study population to healthy women with single pregnancy, we were able to eliminate several well-established confounding factors. Collection of maternal and child biological samples made it possible to use biomarkers as a valid measure of exposure and notification of any changes in its level. Moreover, the outcomes of interest have been assessed by widely used measurements with a standard protocol, which allows for cross-cohort analyses. In addition, among the 7-year-old children, a reliable assessment of the outcomes of interest based on skin prick tests and pulmonary function tests was selected.

The REPRO_PL cohort is of medium size. The response rate in Phase I of the study for all the scheduled visits amounted to about 60%, which is similar to the one observed in the Generation R study in Rotterdam [28]. This is a typical participation rate for the cohort studies with such frequent contacts and detailed measurement protocol throughout the scheduled visits. However, considering withdrawal from subsequent visits this increases the risk that the sample size at this stage will be further limited. The research team needs to make even more effort to increase the participation response in Phase III of the REPRO_PL study. To increase the interest of mothers in their child participation in health and psychomotor development examination, the day and time of the visits are agreed with the mothers. After the examination, all mothers receive results of skin prick and pulmonary function tests, the detailed description of their child’s health, psychological development status, nutritional status and recommendations for a further examination or treatment if needed.
Based on our experience, participation rate in a subsequent examination (data for 1 and 2 year-olds assessments) was about 60%. During the 5 years that have passed from the last examinations of children, the mothers could have changed the address and/or telephone number, and we might not be able to reach them. There is also a possibility that the mothers who were sent the questionnaires by post (500 mothers) would not answer. Therefore, in Phase III we may expect a response rate at the level of 50%. The research team has contact numbers to all the cohort participants (and their close family members), so the mothers may be contacted by phone if needed. Checking questionnaire data and quality of the collected biological samples regularly allows for the identification of any missing or incorrect data, and an additional collection or improvement. In addition, at the stage of analysis, some unexpected problems (lack of some data or information about confounding variables) may arise. In such a case, there is a possibility to update the missing data by means of a telephone interview with the participants.

It should be mentioned that in some cases (when the difference between fluid and crystallized intelligence is too large) general intelligence quotient (IQ) could not be calculated. Moreover, our sample size may be too small to conduct some analyses, therefore, the combined analyses based on the data from the existing cohorts are planned. Finally, although we have data on the assessments of biomarkers for some exposures (ETS, phthalates, vitamins, trace elements for pregnancy period and first 2 years of life) for others, such as alcohol consumption during pregnancy, we need to rely only the questionnaire data which may create bias.

**Potential for collaboration**

Detailed data available from the REPRO_PL cohort and a biobank that we created based on the collected biological samples provides for a unique opportunity for a cross-cohort collaboration addressing important aspects of children’s health and development. This paper provides useful information on the characteristics and biological samples available within the REPRO_PL cohort.

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