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NINFEA project: study protocol

TITLE: "A web-based Italian birth cohort: NINFEA Project (Nascita e INFanzia: gli Effetti dell'Ambiente)"

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1. Introduction

The fetal and perinatal periods have been indicated as important time windows for the life course health, as exposure acting during these periods can modify health during childhood, adolescence and adult life. This is due to the fact that there are specific sources of exposure (lactation, for example), an intense physiological development and different metabolic capacities that make the organism more susceptible to certain exposures. For example, several studies (1-7) have found that fetal development and maternal lifestyles are associated with the risk of cardiovascular disease, diabetes, certain cancers, impaired cognitive function, infertility.

Studies on early risk factors were initially conducted in the Scandinavian countries, thanks to the availability of high quality maternity records and the possibility of link these with other population registries. The limitation of register based studies is that they do not allow to obtain ad hoc information on risk factors, and that they lack of biological samples. Recently, both in European and in non-European countries, cohort studies in which the subjects, with 1 the collaboration of their mothers, are enrolled at the time of birth and the information on the exposures collected already during pregnancy (birth cohort studies) have been established (8-9). Additional data are collected at different times of infancy/childhood. Moreover, blood 2 samples are obtained, both from the mother and the children (eg from the 2 umbilical cord at the time of birth). Finally, the children are followed up to adulthood for the assessment of the outcomes of interest.

Such birth cohort studies allow to evaluate prospectively a large number of etiological hypotheses. However, they have obvious organizational difficulties and extremely high costs. There is therefore the need to hypothesize and test methods that simplify the implementation of birth cohort studies and allow greater diffusion. It is our opinion that the internet is one of these methods.

2. Objectives

The NINFEA project (Nascita e INFanzia: gli Effetti dell'Ambiente) is a birth cohort study that recruits participants via the internet. Studies of this type are intrinsically "multipurpose" and in general they aim at:

1. Investigating environmental and genetic risk factors for maternal complications during pregnancy, such as hypertension and diabetes;
2. Investigating environmental and genetic risk factors for intrauterine growth restriction/problematic fetal development (with indicators such as low birth weight, pre-term birth and low weight for gestational age);
3. Studying the etiology of congenital anomalies; 4. Identifying exposures and individual and genetic factors that increase the risk of occurrence of certain diseases / conditions during childhood and adolescence, such as asthma and obesity.

The study aims to recruit between 10,000 and 20,000 pregnant women and their children, and, through international collaborations, to participate in birth cohort study consortia.

3. Pilot study

We started a pilot study in July 2005 in the city of Turin (900,000 inhabitants). As part of the pilot, we constructed the study website, designed posters and leaflets, and developed three 2 online questionnaires to be completed during pregnancy and 6 and 18 months after giving birth.

The study was advertised in posters and leaflets, and 1200 pregnant women had participated by the end of December 2007. Approximately 90% of the first 800 women have completed the second questionnaire. In July 2006, we surveyed women giving birth in the main obstetrics hospital of Turin to estimate the proportions with access to the internet and awareness of the study in the target population. Of 122 interviewed women, 66% had access to the internet and 42% were aware of the study. Eight women (6.5%) were members of the 3 cohort. In January 2008 we started advertising in websites and forums. We estimated that about 15 women participate in the 2 weeks after the message is posted in a forum.

4. Methods

4.1. Population

The cohort includes babies born to pregnant women who have enough knowledge of the Italian language and the use of the internet to complete online questionnaires, who become aware of the study and volunteer to participate. Participants register on the study website (www.progettoninfea.it) and their children are followedup until adult life. In addition, mothers are followed-up for complications in the late pregnancy and health status in the first years after delivery.

4.2. Recruitment

We use both “active” (offline) and “passive” (online) approaches to advertise the study. Active recruitment involves advertising through standard methods (leaflets, posters, presentations at predelivery classes, etc.) in selected areas in Italy, through collaboration with local centers. Methods of advertisement are developed locally. Passive recruitment is managed centrally. We post information about the study in Italian discussion forums on medical topics, websites dedicated to pregnant women, websites of the main Italian hospitals and Italian associations for diseases.

4.3. Questionnaires

Three online questionnaires (during pregnancy and 6 and 18 months after birth) were developed during the pilot phase on the basis of analogous questionnaires used in other birth cohorts. They obtain information about the mother and/or the child on environmental exposures, reproductive factors, medical history, anthropometrical measures, diet, supplements, health status in the first years of life, cognitive development, and several other factors. Each questionnaire requires about 30 minutes to be completed. The questionnaires have been reviewed at the end of the pilot phase on the basis of the answers of the first 1000 women and will be reviewed periodically, according to emerging research questions.

4.4. Biological samples

At the end of the second questionnaire, women are asked to donate a biological sample. Compliant women receive by mail two kits (OrageneTM), which have been specifically developed for collection of 2 ml of saliva samples from adults or infants, the information sheet and two versions of the consent form, one for the mother and one for the child. Using a prepaid envelop, women mail the kits back to the Cancer epidemiology Unit of Torino, which is responsible for the collection and storage of biological samples. The kits may remain at room temperature for a long 4 time. DNA is extracted from saliva and stored at -80° C for future analysis of individual susceptibility polymorphisms.

At the 19-year questionnaire, participants who are now adults are asked if they are available to participate in the collection of a saliva sample whose objective is to explore environmental exposures and the related influences on the human epigenome. If the answer is yes, they will be sent the material for collecting a saliva sample, containing: i) the information, ii) the informed consent form, iii) the kit for collecting the saliva sample (marked with an ID code) with the attached instructions, iv) two different pre-stamped envelopes (one for the consent and one for the collected sample) to be returned to the Epidemiology Unit, Department of Biomedical Sciences, Via Santena 7, 10126 – Turin.

To guarantee the protection of the privacy and anonymity of the participants, both pre-stamped envelopes have no sender: one envelope is reserved for informed consent, completed and signed; in the other envelope the participant inserts only the kit containing the collected saliva sample. The identification code associated with the kit is different from the one used within the NINFEA project; the association between the two ID codes is maintained in the study database.

In the event that a sample already collected in the first years of life is available, a second objective is to compare the epigenetic markers of the "new" sample with those previously acquired. The saliva and extracted DNA are stored in the TESEO biobank of the Department of Medical Sciences of the University of Turin.

4.5. Follow-up

Active follow-up is managed centrally by contacting women via e-mail, telephone or post and inviting them to fill in the questionnaires following the third. There are short follow-up questionnaires that last 5-10 minutes. In addition to the 4-year-old questionnaire on cognitive development, the 7-year issue on respiratory health, and the 10-year questionnaire on various health outcomes, the 13-year-old is expected to detect some aspects of child health, such as wheezing, diagnosis of asthma, allergies, migraine and headache, including questions related to school learning, anthropometric and pubertal development, some psychological aspects, and lifestyles. To guarantee the validity of the information collected, some questions (musculoskeletal pain and sports) are addressed directly to the child, who is invited by the mother to answers these questions. To present the NINFEA project to children, a dedicated web page is active: NINFEAteen (https://teen.progettoninfea.it). 4 The same method of involving the children (a few direct questions at the end of the questionnaire on the following topics: eating habits and behavior, sleep, screen-time, sport, environment and climate change, social relations and relationship with oneself) is adopted for the 16-year-old questionnaire. In the 16-year-old questionnaire mothers are also asked to provide their children's contact details (email and mobile phone) so that they can be directly involved once they turn 18 years. The 18/19-year-old questionnaire will be submitted to them in a totally untied and autonomous manner from their mothers, thus officially giving birth to the 'second generation' of the NINFEA cohort. A form containing an informed consent information is submitted to the children.

The NINFEA project intends to deepen the study of the health effects of exposure to electromagnetic fields. For this purpose, specific questions were formulated to be proposed twice, one and a half years apart, to the mothers of the participants in the 10-13 age group. The first date is at 10 and 11.5 years, and the second, respectively, at 11.5 and 13 years. Since the 10 and 13-year follow-up questionnaires already exist, the new questions will be added to the current questionnaires, making it only necessary to open a new follow-up window at 11 and a half years. It is also envisaged, for a group of residents in the Metropolitan Area of Turin, the use of a triaxial wrist accelerometer and the compilation of a diary by the child for the duration

of a week in order to collect further data relating to the physical activity and sleep. The funding for this additional information collection derives from the participation of NINFEA in the international project GOLIAT, since July 2022, under the Horizon Europe program of the European Union (grant agreement n. 101057262) which aims to coordinate and promote the research of cohorts of newborns in Europe on electromagnetic fields and their effects.
The NINFEA follow-up continues with the 19-year questionnaire which involves the direct involvement of boys and girls who are now adults (whose contact details were left at the 16-year questionnaire by their mothers) who are asked questions relating to: quality and styles of life, education and work; mothers in the cohort who wish to continue participating in the project are also subjected to a new questionnaire aimed at collecting data relating to: quality and lifestyle, psycho-physical health.

Newborns and their mothers are also followed-up using health related databases, such as the Birth Register, the Hospital Discharge Register, etc. The linkage will be carried out in collaboration with the Regional or National Institutions who are in charge of the Registries.

4.6. Sample size and statistical analysis

The expected sample size is at least 10,000 newborns. Several cases of relatively common diseases are expected to occur in this cohort, such as 600 cases of retarded fetal growth, 1000 cases of asthma, 600 cases of wheezing during the first 18 months of life, 1200 cases of atopic dermatitis, 500 cases of hypertension during pregnancy and 500 cases of childhood obesity. Data will be analyzed using a standard nested case-control approach. The nested studies will be hypotheses based and will investigate gene-environmental interactions when 5 applicable. We will also carry out analyses following a life-course approach, such as those based on joint models in which baseline, intermediate and distal variables can be specified.

4.7. Health promotion interventions

The use of the internet gives the possibility to nest in the cohort and evaluate health promotion interventions. Since July 2007, current smokers who participate to the pilot study are randomized to receive information on the closest centers for tobacco cessation. This intervention will apply also to participants in the enlarged cohort. The main endpoint is smoking 6 and 18 months after delivery. 5 We will evaluate if it is possible to start analogous interventions and deliver them to the members of the cohort.

5. International collaborations

The study is and will be linked with other "traditional" birth cohorts to analyze rare events and exposures. In additions, the same methodology (use of the internet for recruitment and follow-up) will be applied in other countries, to create and international network of web based birth cohort studies.

6. COVID-19 questionnaires

All participants in the NINFEA Project are invited to fill in a short online anonymous questionnaire in April 2020 which aims to investigate COVID-19 the households, with particular focus on children. The questionnaire concerns: influenza-like symptoms in family members, diagnosis of COVID-19, perception of general knowledge about the COVID-19 pandemic, and sources from which the participating women get updates and information about COVID-19.

At the end of 2020, women are invited to complete a full follow-up questionnaire that aims to study: (i) SARS-CoV-2 testing (ii) diagnosis of COVID-19, (iii) occupational and socioeconomic status, (iv) stress assessed using the Perceived Stress Scale (PPS) questionnaire, (v) problems in the management of potential chronic diseases of the child, (vi) strengths and weaknesses (abilities and difficulties) of children assessed using Strengths and Difficulties Questionnaire (SDQ). Similar studies are underway in various European cohorts of newborns with which strategic coordination is envisaged to harmonize the variables collected and future analysis protocols.

7. Update of the protocol

This updated version of the study protocol is available both in Italian and in English at the study website www.progettoninfea.it.

8. Ethical issues

The study has been approved by the Inter-company Ethical Committee AOU City of Health and Science of Turin - AO Ordine Mauriziano - ASL Città di Torino, Turin Italy. A copy of the informed consent and information about ethical issues are available at the website: www.progettoninfea.it.

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