

Causal pathways for asthma (CASPAR) – MoBa “a happy hunting ground”

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ABSTRACT

Large population-based research cohorts, together with national health registries and biobanks are core components in a modern infrastructure for knowledge. Along with the other Nordic countries, Norway has unique opportunities for high quality research based on cohorts, biobanks and registries. Cohorts, biobanks and registries provide a basis for discovering causes and mechanisms of disease as well as for following the development of disease, effects of treatment and consequences of disease. The purpose of this manuscript is to give a brief outline of a project that makes use of these unique opportunities by using data from The Norwegian Mother and Child cohort study (MoBa) to address research questions of common interest, and thus encourage other research groups to use this “happy hunting ground”.

The present study, Causal Pathways for Asthma (CASPAR) funded by the Research Council of Norway, is a subproject of MoBa. The project is designed to take advantage of the potential for research on human biological material in biobanks, by coupling analysis results with data from health surveys, health registries and the health services. The present study is based on an ongoing collaboration between the Norwegian Institute of Public Health (NIPH), the National Institute of Environmental Health Sciences (NIEHS) in the US and several other national and international collaborators within The MoBa Asthma Group. The main aim of the study is to examine a number of hypothesis regarding in utero and early life exposures in relation to the development of different phenotypes of asthma and allergies in childhood.

For the majority of children who become asthmatic and allergic, the differentiation of their immune system into an atopic phenotype probably begins before birth and is established within the first six years of life. This study will advance knowledge of the mechanisms whereby diet and environmental exposures influences gene expression to alter risk of atopic disease. The prime purpose is to build up new knowledge on asthma pathogenesis. There is a great need to develop a new paradigm of disease pathogenesis that takes advantages of applied molecular approaches to asthma and atopic diseases as it occurs in humans at different stages of development. This project takes advantage not only of the basic MoBa samples and infrastructure including links to other national health registries, but also a new national supplementary study of MoBa that includes measures of prenatal exposures in maternal plasma believed to have epigenetic influences on asthma/atopy development and data on genome wide methylation of cord blood DNA.

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INTRODUCTION

Large population-based research cohorts, together with national health registries and biobanks are core components in a modern infrastructure for knowledge. Along with the other Nordic countries, Norway has unique opportunities for high quality research based on cohorts, biobanks and registries. Cohorts, biobanks and registries provide a basis for discovering causes and mechanisms of disease as well as for following the development of disease, effects of treatment and consequences of disease. The purpose of this manuscript is to give a brief outline of a project that makes use of these unique opportunities by using data from The Norwegian Mother and Child cohort study (MoBa) to address research questions of common interest, and thus encourage other research groups to use this “happy hunting ground”.

Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring

symptoms, reversible airflow obstruction and bronchospasm. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath. The development of asthma is most likely triggered by complex interactions between genes and environment. The diagnosis is based on patterns of symptoms, response to therapy over time and spirometry. Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic) (Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2014, <http://www.ginasthma.org/>).

The MoBa Asthma Group has already from 2007 to 2014 published several papers based on data from MoBa. These publications are the basis for the present study Causal Pathways for Asthma (CASPAR) funded by the Research Council of Norway. It is hard to understand the underlying causal mechanisms for asthma. Thus we have focused our work on estimating the associations between different in utero exposures and different asthma related phenotypes including

early respiratory tract infections.

We found that there was no association between maternal alcohol intake during pregnancy and the risk of asthma in the off spring¹. In contrast, higher maternal mid-pregnancy level of vitamin D classified as a 25-hydroxyvitamin D level was associated with a modestly reduced risk of recurrent lower respiratory tract infections (LRTIs) by age three years, but was not associated with current asthma². Furthermore, folic acid supplements in pregnancy were associated with a slightly increased risk of wheeze and lower respiratory tract infections up to 18 months of age³. Maternal folat levels also tended to be associated with an increased risk of asthma at age three⁴. These results suggest that methyl donors in the maternal diet during pregnancy may influence respiratory health in children consistent with epigenetic mechanisms. We found no association between oral contraceptive pill use before pregnancy and respiratory outcomes in early childhood⁵. In contrast, maternal smoking in pregnancy was associated with an increased risk of acute otitis media⁶, lower respiratory tract infections and wheezing symptoms during early childhood⁷. We have further reported that the risk of wheeze increased linearly with maternal BMI in pregnancy⁸. With regard to mode of delivery, results from MoBa indicated that children delivered by caesarean section had an increased risk of developing asthma⁹. Finally, participating in baby swimming may also be related to later wheeze among children¹⁰.

In conclusion, we have suggested that several exposures during pregnancy and early childhood may be associated with early respiratory health outcomes. However, asthma is a heterogeneous disease. Consequently, we need a better and more specific definition of asthma and asthma related phenotypes in school age to suggest that any of these exposures have an effect on childhood asthma. In the present study, CASPAR, we will thus take advantage of the basic MoBa samples and infrastructure, including links to other national health registries such as the Medical Birth Registry of Norway (MBRN) and the Prescription registry (NorPD), and combine this information with measures of prenatal exposures in maternal plasma believed to have epigenetic influences on asthma and atopy, and data on methylation of cord blood DNA to contribute to build up knowledge on asthma pathogenesis.

Epigenetics

In simplified terms, epigenetics is the study of mechanisms that will switch genes on and off. The term refers to heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells terminally differentiate to end up as skin cells, liver cells, brain cells, etc. Epigenetic change can also

have more damaging effects that can result in diseases like cancer. At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA) associated gene silencing is currently considered to initiate and sustain epigenetic change. New and ongoing research is continuously uncovering the role of epigenetics in a variety of human disorders and fatal diseases.

Epigenetic modification of DNA is emerging as a likely mechanism contributing also to the development of asthma and atopic phenotypes^{11,12} and provides as a plausible mechanism for effects of maternal diet on disease risk in the offspring. Mechanisms influencing methylation of DNA are complex and just beginning to be uncovered. Methylation, the best studied mechanism of epigenetic modification¹³, regulates protein activity and gene transcription¹⁴. It has been shown that dietary methyl supplementation in mice increases CpG methylation of offspring DNA¹⁵ and silences certain genes¹⁶. The best established example is the Agouti mouse model in which maternal diet influences both coat colour and obesity in the offspring by means of hypermethylation of the Agouti gene¹⁷. It has also been shown that smoking during pregnancy is associated with DNA methylation in newborns¹⁸⁻²⁰. NIPH and NIEHS have currently investigated the effects of maternal diet in pregnancy on asthma and atopy related diseases in the offspring up to age 3 years, and the findings indicate an association between maternal use of supplements in pregnancy and asthma and atopy related diseases in early childhood^{3,4}. Other nutrients linked to respiratory disorders include folat, fish oil, and vitamins A, C, E and D^{3,4,21,22}.

AIM

The aim of the present study is thus to examine a number of hypothesis regarding in utero and early life exposures in relation to the development of different phenotypes of asthma and allergies to advance knowledge of the mechanisms whereby diet and environmental exposures, influences gene expression to alter risk of atopic disease.

Objective 1: What is the association between exposures during pregnancy, and the development of asthma/atopy in the offspring after age 7 years?

Objective 2: What is the association between different exposures during pregnancy and cord blood DNA methylation?

Objective 3: What is the association between cord blood DNA methylation and asthma/atopy after age 7 years?

Objective 4: Is an estimated association between in utero exposures mediated by child's level of the same exposures?

MATERIAL AND METHODS

The study uses three different study populations to address the research questions of interest.

Study population I (N= 114 000) includes MoBa

participants with questionnaires filled out in the 18th, 22nd and 30th gestational week, when the child is 6, 18, and 36 months, in addition to 7 years. Information gathered includes demographic characteristics, maternal and child morbidity, child development, environmental exposures and diet. *Study population II* ($N=3500$) includes a subsample of MoBa with measures of maternal biological levels during pregnancy of different dietary and environmental exposures. *Study population III* ($N=1800$) includes a subsample of study population II where DNA methylation of cord blood has been analyzed.

Data from MoBa are also linked to the Medical Birth Registry of Norway (MBRN), to obtain information on birth outcomes gathered at the time of delivery, in addition to the Prescription Registry (NorPD), with data on prescription drugs dispensed from all Norwegian pharmacies since 2004.

Variables

The main outcomes are: 1) Asthma, 2) Asthma related phenotypes, 3) DNA methylation. The main exposures are: 1) Maternal dietary and environmental and health related exposures during pregnancy, 2) maternal plasma levels of dietary and environmental exposures, 3) DNA methylation, 4) measures of levels of several dietary and environmental exposures in the children. Possible confounding factors and other factors related to the different research questions will be included in the analyses.

Analyses of biological samples: Bevitallaboratory in Bergen (www.bevital.no) have analysed levels of methyl donor nutrients, vitamins and metabolites in samples from MoBa mothers included in study population II. DNA methylation is measured at 485,577 cytosine positions (CpG sites) in cord blood using the Illumina Infinium HumanMethylation450 BeadChip in study population III (Bibikova et al. 2011; Sandoval et al. 2011).

ORGANIZATION

The present study is based on an ongoing collaboration that is established between the Norwegian Institute of Public Health (NIPH), the National Institute of Environmental Health Sciences (NIEHS) in the US and several other national and international collaborators within the MoBa Asthma Group. The group has high scientific and organizational competencies, and represents several large institutions with an infrastructure that makes this study possible. The core study, MoBa, has its own organization and an extensive infrastructure (www.fhi.no/tema/morogbarn).

PUBLIC HEALTH RELEVANCE

The proposed study significantly enhances our understanding of the complex aetiology of asthma and atopy in childhood. Our study will contribute with substantial new knowledge to the international childhood asthma and atopy research. The information will be important

in targeting health education and health promotion programs and useful for public health institutions and government offices involved in decision-making regarding public health recommendations. Several countries are, for example, discussing fortification programs, and results from this study will add aspects to the discussion. Both negative and positive results will contribute to the understanding of how micronutrients and other exposures affect metabolism and DNA modification. The publication plan includes scientific publications, to be published in high impact peer-reviewed scientific journals. All results will also be published on the websites of NIPH (www.fhi.no) and MoBa (www.fhi.no/tema/morogbarn). Results will also be included in specific newsletter to the participating mothers, fathers and their children.

ETHICAL ASPECTS

MoBa is previously approved by the Norwegian Data Inspectorate and recommended by the Regional Ethical Committee (REK). The linkage of MoBa to the MBRN and NorPD has the same approvals, and the women are informed of these linkages. The women are informed that they can withdraw at any time. The present study has also an approval from the Norwegian Data Inspectorate and recommended by the REK for this specific sub project, a new national supplementary study of MoBa, that include measures of prenatal exposures in maternal plasma believed to have epigenetic influences on asthma development and data on methylation of cord blood DNA and a follow-up of the children to age 7 years. Children are particularly important to ensure high quality care. The specific focus will be to develop and implement standards for epigenetic studies including children and other participants in population-based cohorts (MoBa), emphasizing communication with participants and the society at large, procedures for ensuring data security, and ethically robust approaches to epigenetic disclosure issues.

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