den norske Mor & barn undersøkelsen

PROTOCOL

The Norwegian Mother and Child Cohort Study

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Norwegian Institute of Public Health

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PROJECT DESCRIPTION

The Norwegian Mother and Child Cohort Study

Summary

In order to achieve better health for mothers and children in the future, we wish to test specific hypotheses about the causes of a number of serious diseases by recruiting 100,000 pregnant women to a cohort study. Possible causal factors will be linked to information obtained from questionnaires, blood samples from mother, father and child, urine sample from mother and medical registries. The Norwegian Mother and Child Cohort Study has multiple endpoints. Primarily those associated with adverse pregnancy outcomes will be studied, as well as diseases affecting mother, father or child. Endpoints will be taken from questionnaires and medical registries. The study will be carried out nationally and researchers with relevant questions will be welcome to participate. No interventions will be undertaken, which means that any conditions that may, potentially, expose the families to disease will not be modified. Both basic and applied research will be undertaken, with projects spanning from molecular genetics to welfare. The Cohort study is now nationwide.

Background

The background for the project is lack of understanding about the causes of disease. The main aim is prevention. This can be achieved by identifying the environmental factors that are the links in the causal mechanism leading to disease. The research has to be specific, and planned in a way that allows concrete questions to be answered. The prevalence of a disease, and the number of causal factors involved, must be taken into consideration. Many people are affected by the serious illnesses with which we are concerned, sometimes at a very young age. All of these diseases are the result of a chain of causal events with many components. There is no contradiction between our understanding of disease as multifactorial (or that health is a multidimensional concept) and looking for specific causes for disease. Much can be gained by understanding the critical points in the causal chain, as experience from the diseases we have been successful in preventing and treating has shown.

The causes of the many diseases and complications that can arise during pregnancy are largely unknown, for example still births or serious congenital abnormalities. We know little about why some births occur prematurely. For many diseases that occur throughout childhood, such as diabetes, autism, cancer, rheumatism and allergy, our knowledge is very incomplete, and treatment of these childhood diseases requires large resources. Many of the complaints and illnesses that occur during pregnancy are also poorly understood. These include nausea, pre-eclampsia, pelvic pain and depression.

Knowledge about the causes of disease (epidemiology) is important for several reasons. Finding specific causes can lead directly to prevention. If we know that a toxic substance or medicament causes damage to a fetus, then by avoiding contact with these substances, we can prevent damage. Similarly, if the damage is caused by an infection, we can give advice in order to prevent infection and develop vaccines. Also, if we know more about the causes of disease here will be less unnecessary anxiety. We have a tendency to blame either ourselves or factors in our lifestyle or diet, when we are ignorant about the causes of disease.

These claims can be tested in the Mother and Child Cohort Study.

Another reason for carrying out epidemiological research is to aid in the development of new medicines. Including mother, father and child in the cohort study will enable us to make effective use of new methods in genetic epidemiology. The transmission disequilibrium test can be used to identify the genes associated with disease. In turn, detailed laboratory work can be carried out which identifies the fundamental metabolic errors underlying disease. Medicines can, therefore, be targeted more efficiently. From this perspective, the cohort study will stimulate basic research in molecular genetics both in Norway and internationally.

It is also important to examine commonly held beliefs concerning the causes of disease. Many aspects of modern society can be sources of anxiety. One recent example is the question as to whether mobile telephones can lead to illness. From a biological point of view this seems unlikely, but without scientific evidence this cannot be refuted. Reduction of unwarranted anxiety is a valuable aspect of the project.

Yet another dimension of the project is to examine quality of life and positive aspects of health. The project can illuminate which environmental factors promote absence of illness and healthy living.

Norway has a social infrastructure that facilitates epidemiological research. We can track individuals and generations over long periods of time. The population is well educated and Norwegians have a long tradition of voluntary participation in responsibly carried-out medical research. In addition, several health registries with high quality data already exist.

The cohort study will be conducted over an extended period of time. Children will be followed up until they are adults, and parents will be followed up over many years. This will enable an investigation into serious adult diseases, such as cancer and cardiovascular disease. For example, all women can be monitored for breast cancer: an illness that is increasing in Norway, especially among the under fifty's. The data collected will enable the testing of many important causal hypotheses.

The project will also supply valuable information relating to the causes of long absences from work during pregnancy. Within the research that is being performed on the Norwegian welfare state system, there has been a lack of individual follow-up studies that include health variables. Which factors are most important in predicting long-term disability? Many women experience that illnesses such as pelvic pain, which start during pregnancy, can affect their subsequent health and ability to return to work.

We believe that if we can contribute to the understanding of even a few of the diseases that we will be investigating, the effort will be worthwhile. The knowledge that is derived will be applicable both to future generations and for mothers and children outside Norway. From a strict philosophy of science viewpoint, we acknowledge that it is impossible to demonstrate that a particular exposure is a cause. It is still correct to use causality models (rather than more diffuse risk models) in order to make the aims of the study more precise and easy to grasp.

Study aims

The study aims to calculate the degree of association between potential causal factors (exposures) and ill health in mother and child.

As the project evolves, new causal hypotheses will arise. Laboratory techniques in the years to come will ensure that improved methods are available for tracing exposures in the biological material. The main data set from the questionnaires, urine- and blood samples will be linked to information about many diseases through other databases. In this way, the main project can be seen to be fundamental to all the subprojects. Many research questions can be answered using the main data set alone, while others will require additional data. If a subproject requires further contact with the study participants in order to collect more data, new consent will be requested.

In addition, the distribution of exposures in the population will be described, and estimates of the incidence and prevalence of many diseases will be made. In the case of some diseases and conditions, natural progression can also be described. No interventions will be carried out as part of the main project.

A number of research questions and proposals for subprojects have been outlined. The breadth of interest in the project is apparent. The many projects focusing on women's health and working conditions during pregnancy will give results relatively quickly. Others, such as those focusing on childhood illnesses, will require follow-up over many years.

Diet, infections, hereditary factors, environmental toxins, medication and exposure to occupational hazards can be mentioned as examples of exposures that are of scientific interest. However, they will not be detailed in this protocol.

Design

This is a cohort study, which involves recruitment prior to the onset of the disease that is to be studied. The aim is to compare the incidence of disease in a group of exposed women/children with a group that has not been exposed, while controlling for other factors which can affect the risk. The main project will provide data regarding disease and exposure variables, which can be analyzed without further modification. Links to health registries (e.g. Medical Birth Registry or Cancer Registry) or exposure registries (National census data) will enable the generation of new data sets, simply and effectively.

Many of the subprojects will be based on the cohort design of the main project. When additional exposure data are required, or blood- and urine samples are being analyzed, the relevant design will be a nested case-control study. This involves identifying a sample of subjects that have developed the disease to be studied, and selecting controls that have not developed the disease, and measuring exposure for both groups.

In addition, subcohorts with particular exposures can be followed in more detail. Also, estimates of the joint effects of genes and disease can be made with the mother-father-child triad design.

Sample

The target population is 100 000 pregnant women who will be recruited between 1999 and 2008. The intention is to continue the project until the goal of 100 000 participants is reached. In Norway, there are approximately 60 000 births annually. We aim to recruit women from every county.

A sample size of 100 000 is required because many diseases are relatively rare and exposures (e.g. certain infections) infrequent. A typical congenital malformation has a prevalence at birth of about 1 per 1000, so 100 cases can be expected. But such malformations are clinically heterogeneous, so in practice, it will be desirable to carry out analyses in subgroups. For some malformations, and for other rare conditions such as childhood cancers, collaboration with the Danish Mother and Child Cohort Study will therefore be indicated.

Four conditions can be seen to lead to a potential selection bias. Firstly, women who choose to terminate pregnancy prior to recruitment to the study or who have a spontaneous abortion will obviously be excluded from the study. Secondly, some women may not wish to participate in the study; thirdly women may withdraw from the study after recruitment and fourthly, women may leave the study because of natural causes such as death or emigration. These selection mechanisms will be described, so that it is possible to examine their likely effect on the study aims. The most important factor affecting selection is probably low recruitment. When calculating associations between exposure and disease this can be critical, but not necessarily. However, low recruitment will commonly bias estimates of disease prevalence, particularly if there is systematic variation according to parents' education or other factors that may be associated with willingness to participate.

Case-control sampling will be based on the number of cases that are found. Normally, two to four times as many control cases are required. These will be randomly selected from the whole cohort. A control group can be used for different groups of cases.

Variables

Exposure variables
An exposure variable is defined as one if it is named in the study aims as a potential causal factor. Many of the questions posed in the questionnaire will either directly or indirectly measure exposure. Similarly, specific blood- and urine factors will be exposure variables when they are analyzed as causal factors. A fuller understanding of the framework for these variables can be obtained from examining the content of the questionnaires Unni: link to questioners and the detailed description of blood- and urine sample collection and storage (appendix 2). Subprojects will also enable the collection of further exposure variables.

Health Variables
A health variable is defined as one that describes or defines a health condition, either from the questionnaire, registry or urine- and blood samples. Normally these variables become effect variables in a cause-effect model, but they can also be exposure variables for other health variables. For example a mother's mental health can be the cause of a child's future psychiatric complaint, and low birth weight can lead to a number of childhood ailments. Many health variables will be collected from links to other health registries or as part of subprojects.

Other variables
The questionnaire will also include a number of other variables which conceptually are neither exposure nor health variables. Some of these will correct for known associations, while others are included because they are of general research interest. Many of the questions relating to diet and use of medicaments are of this type.

Table 1
Examples of exposures and health outcomes to be investigated in the Norwegian Mother and Child Cohort Study.

Examples of exposures	**Examples of diseases**
Medication	Pelvic pain
Hereditary factors	Congenital malformations
Infections	Stillbirth
Dietary factors	Premature birth
Environmental toxins	Cancer
Physical activity	Diabetes
Work situation	Asthma/allergy
Occupational hazards	Rheumatism
Interpersonal relationships	Depression
Personal habits	Breast cancer

Data collection

Recruitment
An invitation for participation in the study is sent to women at their home address. The majority will receive this package three weeks before attending routine ultrasound examination in the 17. -19. th week of pregnancy. Names and addresses are obtained from the clinics that have received a request for ultrasound examination, either from a doctor or the woman herself. Each week the hospitals send a list of all women who have appointments for ultrasound examination to the Norwegian Institute of Public Health (NIPH), Medical Birth Registry (MBR) in Bergen. This list includes names, addresses and national identity number, as well as the date of the appointment (appendix 1). Permission to maintain such a list of names has been granted by the Norwegian Data Inspectorate. This list is used by the clinics to prepare labels, expected number of blood- and urine samples and also as a means of calculating the rate of non-participation.

The invitation, which is sent out in collaboration with each participating hospital, describes the purpose of the study, protection of privacy and practical details. It is emphasized that participation is voluntary, and that consent from children will be sought when they are older. The women are also notified that they can withdraw at any time. In addition, women are informed that additional invitations may be received requesting participation in subprojects. This may entail the collection of further data. Information brochures about the project together with Questionnaires I are enclosed. Also enclosed is a consent form, which requires a signature, and a return-paid envelope.

If a woman wishes to participate, the questionnaires and signed consent form are returned either by post to the NIPH, MBR in Bergen, or handed-in at the ultrasound examination.

Ultrasound examination
At this examination, a midwife informs about the project and asks the woman if she wishes to participate. After obtaining consent, urine- and blood samples are drawn. Blood samples are also taken from the participating fathers. Blood- and urine samples are sent the NIPH in Oslo. See appendix 2. If the mother and father are uncertain about participation, they can either consult the midwife or she may refer the couple to a project colleague who can clarify any misunderstandings. Two weeks later all women are sent news from the study as a single reminder.

A copy of the standardized ultrasound form where values can be entered and any abnormal findings noted is sent from the hospital to the NIPH, MBR in Bergen.

Prenatal questionnaires
During the 22th and 30th week, the woman is sent new questionnaires (Questionnaire II and III) with a reply paid envelope. If necessary, a reminder is sent after three weeks.

At Birth
Soon after birth, a blood sample from the umbilical cord and a second sample from the mother are taken. Both are sent to the NIPH in Oslo.

Postnatal questionnaires
Further questionnaires are sent to the mother when the child is six months, eighteen months, three years and seven years old.

Data storage

Person identifiable data
Women who agree to participate are registered in a data base containing name, national identification number, a code number which corresponds to other files, information relating to when questionnaires or blood- and urine samples are sent and received, and whether reminders have been sent. No other information is stored in this database. All information about father and child will be linked to the woman, who is the index person in the project.

Questionnaires
Data files that contain information from the questionnaires and the ultrasound investigation have a code number. This code number, which is the same as the one in the person database, will only be used together with the national identification number in the event that links are to be made to other health registries or data is to be extracted for case-control studies. The questionnaire databases will be stored and checked at the Medical Birth Registry in Bergen. For content Unni link to questionnaires.

Collaboration with Medical Birth Registry (MBR)
Data from the standard notification form to MBR will be included in the database for the Norwegian Mother and Child Cohort Study. The Norwegian Data Inspectorate has granted a concession for this. This is an important link, as it will prevent the project contacting the parents of children who have died at, or soon after birth, as well as allowing the identification of multiple births. Case control studies that are directed towards the parents of children who are born with congenital abnormalities or other specific pregnancy outcomes will also be possible.

Biological samples

The aliquoted blood and urine samples will be frozen to -80^{o}C and stored in freezers at the NIPH in Oslo. Extracted DNA will be stored at -20^{o}C. From 2005 RNA is taken from the cord blood. TEMPUStubes added RNaseinhibbitor stores at -80^{o} C. A specially constructed data application will be used to locate the blood- and urine samples. These can only be removed with a concession from the Norwegian Data Inspectorate.

Database links
All links to databases other than MBR should be approved by the Norwegian Data Inspectorate.

Table 3
Flowchart for data collection

What happens:			
Week/mth	**Hospital**	**MBR**	**Inst.of Public Health**
10-14	Before ultrasound		
	Receive names and addresses of pregnant women generally from referring GP.		
	A copy of list sent to MBR each week.		
	Send information by post to women	Send invitation by post to women with questionnaire 1, one questionnaire for the partner and consent form for both.	
		Receive consent form and questionnaires from participating women and partners	
17	**Ultrasound examination**		
	Women are asked if they will/will not participate		
	Blood- and urine samples taken		
		Receive copy of standard ultrasound form	Receive blood- and urine samples from women and partners
	Later in pregnancy		
18			
22		Questionnaire 2 sent out	
30		Questionnaire 3 sent out	
		Reminder sent after 3 weeks	
	Birth		
	Blood samples from mother and umbilical cord after birth		
			Receive blood samples from mother and child
	Age 6 months		
6 mth		Send out questionnaire 4	
	Age 18 months		
18 mth		Send out questionnaire 5	
	Age 36 months		
36 mth		Send out questionnaire 6	
	Age 7 years		
7 years		Send out questionnaire 7	

Pilot surveys

A small-scale trial survey was completed and the questionnaires and routines for taking and sending blood samples were tested. The overall experience was positive. Physicians working in primary health care collaborated in this study, but after discussion within the Norwegian Medical Association recruitment by this channel was abandoned. From summer 1999, recruitment has been as described above. In November 2005 it was 60 000 women recruited. The overall participation rate is about 40% of all invited women. Estimated total recruitment 110 000 pregnancies and 90-95 000 women by the end of 2008.

In September 2000, a pilot study was started at the Hospital of Bærum with inclusion of the fathers. The reason for this is that occupational hazards, ill health and use of medications by the father may potentially cause mutations in reproductive cells, which can lead to illness in the child. Fathers were asked to complete a questionnaire and give a blood sample. The fathers are now included in all parts of the country. 80% of the fathers participates when women participates.

The project will not be able to require that all the participating hospitals follow exactly the same routines in recruitment, ultra sound examination and the taking of blood-and urine samples. Therefore, the project must be flexible and able to adapt to local conditions. Special support has, for example, been given to the midwives working at some hospitals and to laboratory staff at others.

The availability of economic resources has determined the study's progress. The project is now recruiting women from all parts of Norway, except form the two hospitals Rikshospitalet and Tromsø Universitetssykehus.

Project Organization

Two teams engaged in perinatal epidemiological research started the project: One consisting of researchers at the Medical Birth Registry and The Department of Public Health and Primary Health Care at the University of Bergen; the other attached to the Section for Epidemiology, Norwegian Institute of Public Health, Oslo. After 01.01.02 both groups are parts of The Division of Epidemiology, Norwegian Institute of Public Health, where the study is anchored (Head: Camilla Stoltenberg). The Director of The Norwegian Institute of Public Health, Geir Stene- Larsen, heads the project technically, financially and administratively. Furthermore, the study has a leader team, consisting of 4 members from the division. The principal investigator is Per Magnus. A governmental committee evaluated the Norwegian Mother and Child study in spring 1998. General practitioners took the initiative for this. In Parliament, a large majority approved the study and the Ministry of Health and Social Affairs requested that the Norwegian Institute of Public Health carry out the study.

In order to carry out the data collection, the study has engaged full- or part time, laboratory technicians/midwives at the main participating hospitals. The period of employment is related to the length of time data will be collected in each region. The project has also employed staff in Bergen to manage the databases at NIPH, MBR in Bergen.

International collaboration

The National Institute of Environment Health Sciences (NIEHS) in the US has signed a contract for 1,2 million dollars, giving them access to urine and blood samples from participating women. The purpose of their study is to examine the effect of environmental toxins on childhood illness. The US National Institute of Health (NIH) is funding part of this project. Together with a group of researchers at The Colombia University, New York, a sub-study of Autism related disorders is conducted (The ABC-Autism Birth Cohort). An amount of $ 13 mill from NINDS (National Institute of Neurological Disease and Stroke Health was granted in 2003 for this study. We collaborate with a group carrying out similar research in Denmark. In December 2000, an international advisory group visited the NIPH in Oslo and gave recommendations.

Funding

The Norwegian Ministry of Health granted the project a budget of NOK 1 million for 1998, 1999 and 2000, NOK 5 million for 2001 and 2002 and 6.5 million for 2003.

Through the Norwegian Research Council, funds have been set aside for research in functional genomic. 50 mill NOK over 5 years has been given to a technology platform for human biobanks and health studies, consisting of the Cohort of Norway (a cohort of 200 000 adults) and the Norwegian Mother and Child Cohort Study. In 2002, 4.5 million NOK was allocated to The Mother and Child Study.

The Confederation of Norwegian Business and Industry (NHO) has given NOK 1 million to the project through its occupational environment fund in 2000 and 2001. Private organizations and non-governmental research organizations are currently considering a number of applications.

Concessions from the Norwegian Data Inspectorate

In October 1996, the project was granted a concession by the Norwegian Data Inspectorate. This was renewed in September 2003. The Director at the Norwegian Institute of Public Health is responsible for the registers. Together with the concession, the Norwegian Data Inspectorate also made recommendations relating to participant information and consent. The concession includes information from the NIPH, MBR and information and blood samples from the father. Meetings and correspondence between the Norwegian Data Inspectorate and the project have lead to, amongst other things, changes in the recruitment procedure.

The project involves collecting sensitive personal information. Much effort has already been, and will continue to be, expended on ensuring that third parties cannot link data from the project to a specific name. We require participants to state name, address and national identity number for two reasons. Firstly, we can communicate with participants during data collection and secondly, we can link data to external data sources, such as hospital records.

Data from the questionnaires, blood- and urine samples will be stored with a linkable code number. The code number and personal identification code will only be linked when required in the course of data collection, and later when links to other databases are made. All the data made available to researchers (internal and external) will be stripped of personal identifiers; that is identification will be possible using the code number, but not directly to the person who has provided that information. As a general rule, the results from analyses of blood- and

urine samples will be transferred back to the central database so that others can utilize them, but this can be evaluated in each individual case. When new data are being collected (in connection with a subproject requiring additional consent), consent shall also be obtained to transfer the data to the main database.

It should be particularly noted that the use of all blood- and urine samples would require approval from the Norwegian Data Inspectorate and the Regional Ethical Committee. All links to external data sources require a concession from the Norwegian Data Inspectorate.

The consent forms, letter inviting study participation and the brochure shall all give information regarding the scope of the study and consequences of participation. Newsletters will keep participants informed. Children will be informed personally about the study when they are 15 years. They will be asked to give active consent to continue in the study when they are 18.

Ethical considerations

"Contract" between participants and researchers
While rights and obligations have not been negotiated, the consent form in many ways resembles a contract, whereby participants agree to donate biological material and information about themselves. In exchange a guarantee is given that the researchers will use the material to study the causes of disease. This involves a fundamental trust on the part of the participants; it will be unethical on the part of the researchers not to utilize the material in the manner intended, or to use it for other purposes. New subprojects, which require active participation (completion of new questionnaires, clinical investigations, evaluation of exposure or new biological samples) beyond what is explicitly stated in the signed consent form, will require new consent. Subprojects that require collection of new data will need approval from Regional Ethics Committees and the Norwegian Data Inspectorate. Participants on subprojects must also give permission that data will be channeled into the main project.

Project value
The purpose of the project is to investigate the causes of disease. Knowledge about the causes of disease can lead to good interventions and further laboratory research which can reveal both the mechanisms that underlie disease processes, and lead to new treatment forms. It is also important to disprove false theories regarding the cause of disease and investigate which factors promote good health and absence of disease.

Potential Harm
No interventions will be undertaken in connection with the project, in the sense that conditions resulting in an exposure will not be wittingly modified in order to prevent disease. Participants will not receive the results of blood tests, or other information about themselves, that they are not already aware of. Participants can be recruited to projects on the basis of disease information (e.g. pelvic pain or incontinence) or pregnancy outcomes about which they are already informed (e.g. congenital abnormalities) or based on geographical location, child's date of birth etc. As a guiding principle, recruitment cannot occur on the basis of lifestyle; for example, smoking. If participants are to be recruited on the basis of findings from blood- and urine analysis, they must previously have given written consent stating that they are aware that they will be informed of the results of the blood- and urine analysis.

Some participants may find some questions offensive, others may find that the scope of the questionnaire is wider than they had expected. The drawing of blood samples can also be experienced as unpleasant.

Conflicts of interest between researchers
The study is a national resource that will be available to all bona fide researchers with a legitimate request for data. If different research groups have similar study aims and wish to use the same data, then flexibility will be encouraged and collaboration sought. The leader group's decision will be final in such matters.

Dissemination of results
Publications will be made according to further guidelines (see Appendix 4) Norwegian public health risk evaluations and advice will be given according to the existent guidelines.

Ethical evaluation
Ethical aspects of the project were discussed in a seminar in 1995. A transcript of contributions at this seminar is available on request (Norwegian only). Further, the project has been evaluated by the Regional Ethics Committees for medical research, Health Region II (REK II). This committee is kept informed of all modifications and has approved, for example, the trial project including fathers. From spring 1997 to spring 2000, the project had its own committee advising and making recommendations to the project's executive and working groups.

Appendix 1: Description of the person database

Introduction
The Norwegian Mother and Child Cohort Study uses an advanced database (tracking system) to register and follow the progress of participants through the various phases of the study. Oracle 7.3 has been used to create and access the database, and the screen formats have been developed using Oracle Forms 5.0 and Oracle Reports 3.0. The computers, in which the tracking system is installed, are connected to the local network at the Medical Birth Registry. To ensure the security of the database, MBR's standard password system is used.

The main menu of the tracking system shows the most important tasks in the Norwegian Mother and Child Cohort Study. The description in this appendix follows these tasks. All the tasks are important, but screen layouts are not shown.

The unit that is followed by the tracking system is the pregnancy, not the woman. This is because a woman can participate in the project during several pregnancies.

Registration of new ultrasound appointments
A list of all women receiving ultrasound appointments is compiled at each hospital. These lists are transferred to the person database at MBR either manually or in encrypted electronic form.

Electronic transfer is direct, while paper lists are keyed in manually. If a woman is already registered in the system, the information will be updated.

Data relating to pregnancies being registered for the first time are entered. This information includes name, address, date, and where the ultrasound will be carried out. If a woman is already registered, either for this or a previous pregnancy, notification will automatically appear on the screen and the date for the new ultrasound can be checked.

A pregnancy being registered for the first time in the tracking system is given the status ***INNREG (Registered).*** The only information about the pregnancy at this stage is the actual registration.

Registration of new ultrasound forms
The hospital sends a copy of the ultrasound form for all women who have agreed to participate in the study. Estimated delivery date and date of last menstruation are entered from this form. These variables are important so that future mailings from the project are sent out at the correct time. In addition a number of parameters are registered (*para*, *ab* and *gravida)* to ensure that individual pregnancies are kept completely separate. A variable noting the number of foetuses is used after the birth to ensure that the correct numbers of umbilical cord samples have been taken. Further information from the ultrasound examination is registered in a separate data file (see appendix 3).

Mailings, reminders and returned forms

Appendix table 1: Mailing overview

Mailing	Timepoint for mailing	Timepoint for reminder	Target group
Questionnaires 1 and 2 + consent form [1)]	approx. 3 weeks before u.s. exam.	at u.s. exam.	women with status INNREG (Registered)
Questionnaires 1 and 2 [1,2)]	after consent form received	3 weeks after mailing	women with status DELTAKER (Participant)
Questionnaire 3	in 30th or 31st week of pregnancy	3 weeks after mailing	women with status DELTAKER (Participant)
Questionnaire 4, 5, 6 and 7, one for each child	When the child is 6, 18 and 36 mths, and 6 years old	3 weeks after mailing [3)]	women with status DELTAKER(Participant)
Consent form reminders		monthly	women for whom samples or questionnaires have been received, but status INNREG (Registered)

1. Women attending ultrasound examination at hospitals where fathers are also being recruited will also receive an invitation for the father containing questionnaires and consent form.
2. Only sent to women who have entered the tracking system from other sources than through ultrasound clinics.
3. Does not apply to triplets who are treated individually.

When questionnaires 1 and 2 are sent out (line 1, table 1), status changes from INNREG (registered) to INVITERT (invited)

The return date for each kind of questionnaire is entered in the tracking system. Consent form reminders (last line, table 1) are only sent to women from whom samples, or one or more completed questionnaires, have been received.

Questionnaires that have been returned are scanned and interpreted by a high capacity Fujitsu scanner and the program Eyes and Hands in Windows NT. This program reads marked and numbered fields and also allows the operator to manually enter data that the program finds difficult to interpret. The questionnaires are scanned and stored as optical images, which are then displayed on the screen as they are read by the program. This allows the operator to add or modify codes when necessary. Each scanned questionnaire is allocated a code consisting of a pregnancy identification number and a number indicating the type of questionnaire. These code numbers are transferred to the person database to keep track of the questionnaires that have been scanned.

Returned consent forms
The consent form is an important item in the tracking system. When returned, the consent date is noted and the pregnancy is given the status DELTAKER (participant). Only pregnancies that have been allocated this status are considered to be part of the study, and will be followed up with further questionnaire mailings.

Change of status
It is apparent from the description so far, that the status of the pregnancy is decisive in allowing progress through the tracking system. As soon as a pregnancy is registered, it is given the status INNREG (registered). Thereafter, and for as long as it remains in the system, it will always have a status. The different types of status are shown in appendix table 2. When a woman, the Biobank or a hospital contacts the project indicating that the status of a pregnancy should be changed, a special command is used.

Appendix table 2 Overview of events which alter status in the tracking system

Status	Event resulting in change of status
INNREG (rgistered)	The pregnancy is registered in the tracking system.
INVITERT (invited)	First mailing to the expectant mother with status INNREG is sent out.
DELTAKER (participant)	Consent form returned.
ABORTERT (abortion)	Notification of abortion either from the woman herself or the hospital.
UTMELDT (withdrawn)	Notification of withdrawal from the study.
VIL SLETTES (wants to be deleted)	Notification of withdrawal and that all data is to be deleted.
SLETTET (deleted)	Notification from the Biobank that blood samples with status VIL SLETTES are deleted.
DØDFØDSEL(still birth)	Notification that the baby died (after 16th week) from MBR or woman herself.

Deleting a pregnancy

A pregnancy can be deleted if a woman having first agreed to participate and later wishes to withdraw and have all the data concerning her pregnancy deleted. The resulting status is VIL SLETTES (to be deleted), and the Biobank is requested to delete blood-and urine samples. Data already registered from questionnaires can also be deleted from the main data files. However, data that have been made anonymous and already given to researchers cannot be deleted.

On receipt of an acknowledgement from the Biobank that blood- and urine samples have been deleted, the pregnancy is again called up and the date for deletion of the blood sample entered. The status of the pregnancy is then automatically registered as SLETTET (deleted). At this stage, the only information remaining in the system is that the woman has participated in the study during the pregnancy. A letter is sent to the woman, stating that all the information about her has been deleted.

Data from the Biobank

Once a week, a computer generated encrypted list of newly registered blood-and urine samples is sent to the project from the Biobank. Data from this list are entered into the tracking system. This enables consent form reminders to be sent and compilations of pregnancies with complete sets of blood-and urine samples to be made.

Linking data from MBR and DSP

Before questionnaire 4 is sent out, data from the NIPH, MBR and National census data (DSP) are linked to the tracking system. This is to confirm the date of birth and ensure that questionnaire 4 is only sent to women having one or more living children in the registered pregnancy. In addition, information about multiple births (twins, triplets) enables an appropriate number of questionnaires to be sent.

Appendix 2: Biobank description

Biological samples will be collected from participants during the Norwegian Mother and Child study (Fig , page 23).

At ultrasound examination: From the pregnant women: Blood- and urine samples
From the father: Blood samples

At and after the birth: From the baby: Umbilical cord samples.
From the mother: Blood samples.

SAMPLING TECHNIQUE
All samples are taken at the hospital and sent to the Biobank at The Division for Epidemiology, Norwegian Institute of Public Health, Oslo for registration, processing and storage.

At ultrasound examination
Samples taken from pregnant women, the K1 sample:
Tube 1: 7-ml EDTA vacutainertube with whole blood
Tube 2: 7-ml EDTA vacutainertube with whole blood
Tube 3: 7-ml EDTA vacutainertube with whole blood
Tube 4: 3-ml EDTA vacutainertube with whole blood
Tube 5: 8-ml urine

Tube 2 is centrifuged in a standard centrifuge (not a chilled centrifuge) and 3-ml plasma is transferred to a 5-ml empty plastic tube.

Samples taken from fathers, the F samples:
Tube 1: 7-ml whole blood (EDTA)
Tube 2: 7-ml whole blood (EDTA)
Tube 3: 3-ml plasma transferred from tube 2 (after centrifugation)

At birth
Cord blood, N:
Tube 1: 10-ml whole blood (EDTA)
Tube 2: 3-ml TEMPUS tube (RNA with 3-ml whole blood)
Tubes are filled as much as possible using aspiration.

Maternal sample, K2:
Tube 1: 7-ml whole blood (EDTA)
Tube 2: 7-ml whole blood (EDTA)
Tube 3: 3-ml plasma in a plastic tube (transferred from tube 2 after centrifugation)

All samples are labeled with personal identification code, together with information on type of blood sample: K1, K2, N or F. This information is also labeled to a form stating where and when the sample was taken.

TRANSPORT
All samples are stored at 4°C until they are sent to the Norwegian Institute of Public Health.

If possible, blood- and urine samples should be sent the same day they have been taken. The samples are sent as ordinary mail or by car delivery from the hospitals in the Oslo area (not on ice or frozen). Samples sent by ordinary mail usually arrives the next day. Information notes are sent together with the samples.

The samples are processed as soon as they arrive at the NIPH. Exception: samples arriving on Saturdays will be stored at 4°C until Monday morning.

THE BIOBANK
A specially constructed data application (the Mother-Child-program) has been made for the Biobank. Its function is to keep track of where each individual sample is stored and ensure that samples cannot be mixed up. The program communicates with the software in a pipetting robot so that all the samples in the pipetting robot are correctly registered in the Mother-Child-program.

Registration of samples:
All in-coming samples are labeled with the woman's name and personal identification code. The samples are registered in the specially constructed program using name, personal identification code and the type of sample (K1, K2, N, F). In addition, the hospital's name and dates for sampling and arrival at the Biobank are registered. The contents of the sample (whole blood, plasma, urine) are also registered, together with comments when these apply, e.g. coagulated umbilical cord sample, too little blood in tube, etc.

On storage, each sample is given a sample code, and the personal identification code is encrypted/protected. Access to the file, which allows the data to be deencrypted, will be strictly limited to authorized personnel.

Cord blood samples:
Umbilical cord blood will not be centrifuged at the hospitals. This will take place in the Biobank at the NIPH, so that a plasma sample can also be obtained.

Aliquoting the samples:
A robot-aided pipetting system is used to distribute whole blood and plasma between 96-well microtiter and deep-well plates. All the plates are mechanically heat-sealed before they are frozen.

Whole blood: 1860-µl is transferred to two deep-well plates (930-µl in each). A single plate will therefore store blood from up to 95 people and at least one control. Storage tp. -80° C. (fig 1).
3-ml whole blood (EDTA) is frozen without any processing

Plasma: A total of 1.8-ml plasma is divided on microtiter plates. 300-µl plasma from each person is stored, for example, in position 1B on six separate plates. Storage tp -80°C (fig 1).

Urine: 5,58 ml urine subdivided and frozen in deep well plates.

DNA extraction:
In addition to whole blood and plasma, DNA is also stored.

DNA is extracted from the rest of the blood, using a DNA extraction kit (FlexiGene, Quiagen). Approximately 150 – 700-mg DNA is obtained from each participant. The critical steps in the procedure, implying a risk that either samples or reagents are accidentally exchanged, are supported and controlled by the computer program. After the DNA is diluted leaving all samples with the same concentration, 100-ng/µl, a robotic system pipettes out 5 aliquots of 1,5-ml into deep-well plates. Storage tp -20° C. (fig 1).

DNA from umbilical cord blood is extracted using the same kit. The samples that have coagulated are frozen at -20°C. During thawing, clotted samples are crushed using a tissue homgenizator before the standard procedure is followed.

Storage:
Each sample has a unique laboratory code and a unique location.

The sample will have a designated location on a plate. The plates are previously marked with two labels with a unique bar code and are placed in a containing rack, which is also marked with unique codes. The freezers also have code numbers.

Samples from each participant are stored in at least two separate freezers, connected to an alarm system.

Retrieval:
When samples are to be retrieved, the starting point is a list of personal identification code. This will be entered into our computer program, and the program will find the correct location for the sample. As all samples are stored in 96-well plates, they will be subject to thaw-freeze cycles each time a plate is retrieved. As certain analytic techniques require that a sample has only been subject to a limited number of thaw-freeze cycles, it is important to keep a record of how often plates have been thawed. Other samples from the same individual will always be available at another location, which has not been thawed. The seals on the plate are only broken for the samples in question, and these are rapidly resealed prior to refreezing.

Deleting samples:
In the event a woman wishes to with-draw the project and that all her data and biological material be removed, a delete function is found in the data program. All information about the woman and her samples is then deleted permanently from the files. Blood samples will remain in the freezer, but no identification will be connected to these.

Communication with the Medical Birth Registry, Bergen:
Once a week a report is sent to MBR listing the samples that have been received at the Biobank. This information is encrypted and sent by electronic mail.

The Norwegian Mother and Child Study, Norwegian Institute of Public Health (NIPH).

Blood specimen at all stages from mother at ultrasound to biobank storage, plan January 2006

Mother at ultrasound	Handling at hospital, before shipment	In the post + at arrival, Biobank	Handling in the biobank, NIPH	Storage, NIPH
7 ml EDTA-blood	7 ml →	1) 7 ml →	7 ml → 2 ml whole blood → 930 µL 5 ml whole blood → 930 µL	Whole blood - 80 °C Plates with 96 wells
7 ml EDTA-blood	Spindown → 5 ml conc. whole blood →	2) 5 ml →	5 ml conc Whole blood → mix → DNA Extraction	5 plates DNA - 20 °C (100 ng/µl/well)
	2 ml plasma →	3) 2 ml →	2 ml → 0.3 ml, 0.3 ml, 0.3 ml, 0.3 ml, 0.3 ml, 0.3 ml	Plasma - 80 °C Plates with 96 wells
3 ml EDTA blood	No processing at hospital, sent directly to biobank in Oslo →	Shipment: normal postage →	3 ml → No handling →	Whole blood - 20 °C Individually labelled tubes
7 ml EDTA blood	→	→	7 ml → 930 µL, 930 µL, 930 µL Spindown, Plasma into individual tubes	Plasma - 80 °C Individually labelled tubes (Matrix plates)
Urine Vacutainer specimen cup	8 ml into tube →	8 ml Shipment: normal postage →	8 ml → 930 µL, 930 µL, 930 µL, 930 µL, 930 µL, 930 µL	Urine - 80 °C Individually labelled tubes (Matrix plates)

Appendix 3: Description of the questionnaire database

Images of the questionnaires are scanned. These raw data files are stored. They make the starting point for coding variables which the scanner cannot manipulate directly, e.g. information about employment.

A database is constructed for each of the questionnaires and the ultrasound form. The databases can be linked using a serial number.

Quality controls will be carried out during and after the scanning process and before final storage.

A working group has been formed consisting of one person from The Norwegian Institute of Public Health in Oslo and one from MBR, who will make detailed instructions for quality control. The working group will ensure these instructions are carried out and that quality control is adequately documented. They will also suggest methods for checking the validity of the data.

Questionnaires having passed through the quality control will be stored in the databases. Each month, a copy of the databases at MBR in Bergen will be sent to The Division of Epidemiology in Oslo. Access to the data and agreements regarding analysis and publication, will be decided by the publications group.

The databases will be constructed and accessed using Oracle. Files intended for use by researchers for analysis, will be in SPSS format with a built-in codebook.

Appendix 4: Guidelines for access to data and publication

Guidelines for access to data and biological samples in the Norwegian Mother and Child Cohort Study (MoBa)(12.05.05).

Purpose of the guidelines

- to contribute to research with high scientific quality
- to contribute to rapid publication of important results from the study
- to contribute to good availability of data for research purposes

Who can apply for access to data?

Researchers who have an interest in using data for research purposes can apply for access to data. Any delivery of data will be considered as a subproject in MoBa. The applicant has to be affiliated with an institution with competence in conducting research projects that is willing to be responsible for a subproject. Inexperienced researchers must have a scientific supervisor belonging to such an institution. All subprojects must have a principal investigator with scientific responsibility for the project. For each subproject, a contract will be written between the Norwegian Institute of Public Health and another institution. A contract gives the right to study one or more research questions for a defined limited period of time.

How to apply for access to data

To apply for access to data in MoBa, the electronic application system for the Norwegian Institute of Public Health should be used. At present the electronic application system has not been finalized and all applications should be sent to Marit Hasle at the Norwegian Institute of Public Health (marit.hasle@fhi.no).

What kind of access is granted

Access is granted to data from the MoBa database. For detailed information about the content of the database please look in the protocol (http://www.fhi.no/dav/D66DB9C31F.pdf). The MoBa database contains questionnaire data filled out by the participants in the study as well as additional data from subprojects and linkages. In addition, biological samples for specific analyses can be handed out according to the conditions mentioned below. Given the extensiveness of the database the hand out of data will be adjusted on a case to case basis. Only data without personal identifiers are handed out. No exclusive rights to the data are given. However, exclusive rights to publish on specific research questions for a limited period of time are granted. Normally the limited time period is three years, but this period may be extended according to the complexity of the subproject. Extension of the limited period can be applied for.

Decisions on applications for access to quality-assured data that already exists in the database can be made without delay. Applications for data that are not yet collected or not in a quality-assured state will, as the main rule, only be registered as an interest, and any application will be treated at a later point in time. There are exceptions from this main rule (see next paragraph).

Commitments

If a researcher wants to apply for economic support from one or more funding agencies in order to conduct a subproject based on data from the cohort, the Norwegian Institute of Public Health can give rights to the researcher in advance. This creates a commitment that will last until the outcome of the application is known. The data collection in MoBa is to a large extent

financed by grants from funding agencies. The Norwegian Institute of Public Health has contracts with several agencies including the Norwegian Research Council, The European Community and The National Institutes of Health in the United States. These contracts limit the freedom other researchers have to perform research in some specific areas.

What should be included in the application?
This is thoroughly described in the electronic database. The applicant should specify the institution, responsible person, co-workers, title and purpose, and include a short description of the subproject. This description should be brief and start with the background for the project and the reasons for the choice of research question. Each research question should be formulated in one sentence with individual sentences for sub-questions if necessary. The dataset to be analyzed should be described. The applicant must specify whether additional data are to be collected from the participants or from other sources, and any use of biological material must be specified. The application should also contain a time schedule, a publication plan, and a budget.

Considerations to be taken into account when access to data is granted
The research question has to lie within the general aims for MoBa according to the protocol. In addition, the following criteria apply:

- Scientific quality and originality
- Scientific environment surrounding the applicant
- Usefulness for preventive or curative medicine
- Scientific, administrative, practical, and economical contributions to the planning and/or the collection of data in MoBa

Additional conditions for access to biological material
As opposed to data from questionnaires which can be reused, biological material is a limited resource. In addition to the abovementioned criteria, the following conditions apply:

- That the project has a research question that cannot be answered by using biological samples from other sources
- That the results from laboratory analyses should be reused for other scientific purposes
- The amount of material to be used
- Documentation from the laboratory that the specified analyses can be conducted with high quality

The choice of the number of samples to be analyzed must be accompanied by a statistical power calculation. It is important that there remains sufficient biological material in the biobank until the children in the cohort have reached adulthood. The samples that are handed out will only have laboratory serial numbers to enable results of analysis and left-over material to be tracked and relocated. When the results of the laboratory analyses have been returned to MoBa, the results, together with other relevant variables (e.g. case/control status), will be sent to the researcher as anonymous files. The data from the laboratory analyses will be available for other researchers 3 years after the results have been sent to the primary researcher

Who decides?
The applications are received and controlled by the staff of the Norwegian Institute of Public Health as part of the continuous management of MoBa. The scientific judgment is provided by an advisory committee of four epidemiologists. The committee is appointed by the

Director of the Norwegian Institute of Public Health. Two members are from Norwegian Institute of Public Health. The two others represent the Universities of Norway and the Regional Health Authorities of Norway. In specific cases, outside experts can be consulted. The decision to accept or reject a proposal for a subproject is taken by the Norwegian Institute of Public Health.

What happens if more than one researcher is interested in the same research question?
In situations where more than one researcher is interested in the same research question, the researchers will be encouraged to collaborate, either on analysis and publication or by dividing the area of interest between each other. In cases where these solutions are difficult, the application that meets most of the conditions mentioned above will be chosen. As part of the coordination process, open scientific meetings around research questions of general interest will be held. These meetings are to be announced broadly.

Applicants from abroad
Applicants working at research institutions abroad must have one or more Norwegian co-workers, and show that the subproject will contribute to scientific development in Norwegian research groups.

Formal permissions
If analyses of biological material are to be performed, the applicant, in cooperation with Norwegian Institute of Public Health, must seek the necessary formal permissions from the Data Inspectorate. In cases where an applicant wants to link data from the MoBa database to another registry or dataset, a concession from the Data Inspectorate is required, sometimes with an exemption from the rule of professional secrecy from the Directorate for Health and Social Affairs. If new data are to be collected directly from the participants, either through questionnaires, interviews, clinical assessments, or as biological material, sub-projects should be well grounded and all expenses for the collection of data must be covered by the applicant. Written informed consent must be collected from the participants, and a concession from the Data Inspectorate and a recommendation from one of the Regional Committees for Research Ethics is required. It is important to ensure that the participants are not overwhelmed with new extensive questionnaires or requests for biological samples.

Financial conditions
As part of the contract, a subproject must pay 100 000 NOK to the Norwegian Institute of Public Health at the time of data delivery. Previous financial and other support will be taken into consideration, implying that this sum can be negotiated. When access to biological material is granted, extra work performed by the staff of the biobank must be covered by the applicant. The expenses for handling samples will be negotiated on a case to case basis.

Rules for publication
Results from subprojects shall not be mentioned or discussed publicly before they have been published in scientific publications or as abstracts. All manuscripts must be reviewed by the Norwegian Institute of Public Health (please send manuscripts to marit.hasle@fhi.no) before they are submitted for publication. MoBa should be properly mentioned and the presentation of data should be in line with the conditions given. The project will have a 14 days deadline for commenting on drafts for articles. All publications must contain the following sentence: “The data collection was conducted as part of the Norwegian Mother and Child Cohort Study at the Norwegian Institute of Public Health”. All articles also have to refer to an article in the

main study describing material and methods. The Norwegian Institute of Public Health is not responsible for the scientific content in articles from external researchers.

The content of the contract

When the application is approved a contract will be sent to the applicant for their signature. The contract will contain the title of the sub-project, the name of the principal investigator and the institution. The research question and the period of time in which the access has been granted must be referred to. For biological material information about relocation of analysis results must be given. After signatures, a dataset from the database in Bergen will be handed out for subprojects that do not require biological samples. Biological samples will be sent from the biobank in Oslo, and datasets will be sent after the laboratory results have been included in the MoBa database in Bergen.

Appendix 5: Consent form

Consent form from mother

for participation in the Norwegian Mother and Child Cohort Study

I have read the letter inviting participation and the information brochure about the Norwegian Mother and Child study and understand that the information I give will be treated strictly confidentially. I am aware that the project has been approved by the Regional Ethics Committee for medical research and by the Norwegian Data Inspectorate.

Participation in the Norwegian Mother and Child Cohort Study will entail the following:

- that I complete questionnaires, during and after pregnancy, about my own and my child's' health and living conditions
- that I give a blood sample and a urine sample, during pregnancy and one after the birth, and that a sample is also taken from the umbilical cord at birth
- that the blood samples from myself and my child will be stored and used in the future in research to study causes of diseases, including heredity. Laboratories in Norway and other countries will carry out this research following approval by the Regional Ethics Committee for medical research and the Norwegian Data Inspectorate that the blood samples can be used for this purpose.
- that the results from ultrasound examinations carried out during the pregnancy will be made available to the project
- that the blood sample which is taken from my child to test for PKU (phenylketonuria) may be made available to the project
- that no results (either concerning my own or my child's health) will be sent by the project to me
- that information about myself and the child can be obtained from other sources, for example the Medical Birth Registry and hospital records, following approval by the Norwegian Data Inspectorate
- that I can be asked to participate in further projects where I will be required to give additional information (or biological samples). Participation will be voluntary, and all such additional projects will satisfy conditions laid down by the Norwegian Data Inspectorate and the Regional Ethics Committee for medical research
- that information and blood samples will be stored indefinitely. This is a long-term study that will also investigate the reasons why diseases occur in adulthood. My child will be informed about the project when he/she is 15 years old, and consent requested from the child that he/she remains in the project when they are 18 years old
- that no information or biological samples will be made available to researchers before name and national identity number have been removed
- that participation is voluntary and that I can withdraw from the study at any time by writing to the Norwegian Mother and Child Cohort Study.

I have read the information above and agree to participate in the Norwegian Mother and Child Cohort Study.

Name: _____

National identity number *(11digits)*:_____

Date:_____ Signature:_____

My address on the invitation letter is wrong, the correct address is:

_____

Informed consent form father

I have read the introduction brochure concerning the Norwegian Mother and Child Study and understand that the information I provide will be handled strictly confidentially. I understand that the study has been considered by the Regional Committee for Ethics in Medical Research and approved by the Data Inspectorate of Norway.

Participation in the Norwegian Mother and Child Study requires the following:

- that I complete the questionnaire concerning my own health, life style and working environment
- that I provide a blood sample at the time of the ultrasound examination
- that my blood sample will be stored in a "biobank" at the Norwegian Institute of Public Health. The sample will have no personal identifiers and will be stored with a code number. The blood sample will be used solely for research purposes addressing causes of illness, including the interaction between genetic disorders and environmental factors. This will be conducted in laboratories in Norway and other countries after the specific use of the blood sample has been considered by the Regional Committee for Ethics in Medical Research and approved by the Data Inspectorate of Norway
- that I will not receive any results concerning my health or from the analysis of my blood sample
- that information about me may be obtained from other sources such as the Medical Birth Register and hospital records following approval from the Data Inspectorate of Norway
- that I may be asked to participate in examinations that require the collection of additional information (and samples). Such sub-projects will be considered separately by the Data Inspectorate of Norway and the Regional Committee for Ethics in Medical Research. Participation is voluntary and is not necessary for continued participation in the main study.
- that the information and blood sample will be stored indefinitely
- that no information or samples are made available to researchers before my name and birth identification number are removed
- that I may withdraw from further participation in the study at any time by writing or calling the Norwegian Mother and Child Study. In addition I may ask that information and blood samples collected be deleted/destroyed without providing a reason.

I have read the information and give my informed consent to participate in the Norwegian Mother and Child Study. (Please write clearly in CAPITAL LETTERS.)

Name:
Birth identification number (11 digits):
Address: Post number:
Partner's name:
Partner's birth identification number (11 digits):
Date: Father's signature:

Please return with the questionnaire.

Please keep the copy for your records.