Data Resource Profile: The Aarhus Birth Cohort Biobank (ABC Biobank)

Lotte Maxild Mortensen,1* Bodil Hammer Bech,1 Ellen Aagaard Nohr,1 Mogens Kruhøffer,2 Søren Kjærgaard,3 Niels Uldbjerg,4 Jørn Olsen1 and Tine Brink Henriksen5

1Section for Epidemiology, Department of Public Health, University of Aarhus, Denmark, 2AROS Applied Biotechnology A/S, Aarhus, Denmark, 3Department of Public Health, University of Aarhus, Denmark, 4Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejby, Denmark and 5Department of Paediatrics, Aarhus University Hospital, Skejby, Denmark

*Corresponding author. Lotte Maxild Mortensen, Section for Epidemiology, Department of Public Health, University of Aarhus, Barhelin Allé 2, 8000 Aarhus C, Denmark. E-mail: lmm@soci.au.dk

Accepted 29 August 2013

Exposure during fetal life may have long-lasting health consequences for the child. Cohorts with biological material are necessary to investigate the possible biological mechanisms behind this potential early programming of disease. The Aarhus Birth Cohort Biobank was established in 2008 as an amendment to an ongoing research database, the Aarhus Birth Cohort. It aims to provide the opportunity to investigate the role of genetic factors, environmental exposures and lifestyles in pregnancy on the risk of disease in the offspring. All pregnant women who plan to give birth at Aarhus University Hospital, Skejby, and the fathers-to-be are invited to participate in the Aarhus Birth Cohort Biobank. Blood samples (mother 54 ml, father 4 ml) are drawn at the time of the routine ultrasound scanning in gestational week 12. At the same time, the women fill out a detailed questionnaire on medical and lifestyle factors. Immediately after birth, blood (10 ml) from the umbilical cord and umbilical cord tissue are sampled. Samples from the mothers are separated into plasma, buffy coat, erythrocyte suspension and serum before freezing at −80°C. Samples of whole blood are also stored, from both the mother and the father. Plasma, buffy coat, erythrocyte suspension and tissue from the umbilical cord are stored at −80°C. All researchers can apply for access to the database. For more details, see www.ab-biobank.dk or tbh@dadlnet.dk.

Background for the data collection

Exposures during fetal life or in early childhood may have long-lasting health consequences for the child. This early programming carries a major unexploited potential for preventive and health-promoting measures. Many countries are now investing large research resources in establishing the infrastructure for running cohorts.1,2 We established a pregnancy cohort biobank: The Aarhus Birth Cohort Biobank (ABC Biobank).

From a global perspective, Danish children are healthy, but the perinatal period is still associated with high morbidity and mortality; approximately 10% of all newborns are admitted to hospital and the mortality during the first year of life corresponds to that of 55-year-old males, in Denmark in 2012 (Statistics Denmark, http://www.dst.dk/en). Researchers have tried to unravel the underlying mechanisms of pregnancy complications and the causes of disease and death in the newborn, but a great deal of unexplained mechanisms remain. Thus, the causes of preterm birth and pre-eclampsia continue to be poorly understood, just as we know little about the health consequences of the significant increase in average

1697
birthweight. An increasing incidence of, for example, autism spectrum disorders, asthma, behavioural and attention disorders and diabetes may be attributed to exposures during fetal life such as infections, diet, toxic compounds or deficiency in vitamins and trace elements. A large number of studies indicate that these exposures interfere with fetal growth and development and may modify disease susceptibility years after birth, but access to biomarkers or genetic factors is difficult. New studies should include more precise measures of exposure and gene expression. Furthermore, to better understand the aetiology of these diseases, we need population studies that cover the time span from conception to adult life.

We decided to establish a biobank, the Aarhus Birth Cohort Biobank, as an amendment to an ongoing research database, the Aarhus Birth Cohort, which has collected data during pregnancy and delivery for women since 1989. The overall aim of the ABC Biobank is to provide the opportunity to investigate the role of genetic factors, environmental exposures and lifestyle in the diseases of newborn babies and children. The focus is on perinatal exposures and diseases studied in a life-course perspective.

Data resource area and population coverage

The ABC Biobank is an ongoing collection of biological material from pregnant women, fathers to-be and newborns that are enrolled in the Aarhus Birth Cohort (Figure 1).

Eligible candidates for the ABC Biobank are all pregnant women who plan to give birth at Aarhus University Hospital, Skejby, the fathers-to-be and their offspring. Information about the ABC Biobank, a questionnaire, a consent form and a postage-paid envelope for response are sent to the pregnant woman in early pregnancy, and she is asked to discuss the matter with her partner. They can register by signing the consent form and flagging the maternity record with a participation label, which they keep for all subsequent antenatal visits and delivery. The pregnant woman can also register on behalf of herself and her child without her partner’s participation. It is possible to participate in the biobank without returning the questionnaire and vice versa. It is emphasized that all participants are free to withdraw from any part of the data collection at any point in time.

At the routine ultrasound scan visit at gestational week 12, one of the biomedical technicians in the biobank contacts the woman or couple, offers additional information and collects the samples. Couples who have not returned the consent form will also be approached and introduced to the project. The couple can then sign the consent form if they agree to participate, and the blood samples will be drawn. At birth, the ‘flag’ on the maternity records will notify the midwife that she is requested to collect the umbilical cord samples immediately after delivery.

Survey frequency

The first samples of biomaterial were collected in August 2008 and, by December 2012, 9000 families had been enrolled. We have blood samples from mother, father and child for 62% of the families and 40 new families are recruited weekly. At present, approximately 5000 babies are born annually at Aarhus University Hospital and the Aarhus Birth Cohort has collected data on 100,000 pregnancies, deliveries and newborns since 1989 (Box 1).

Figure 1 Data collection in the Aarhus Birth Cohort and the Aarhus Birth Cohort Biobank.

1The pregnant women complete the questionnaire in the second trimester.
2Blood samples are collected at routine ultrasound scanning in gestational week 12.
3The midwife attending the birth completes a detailed birth registration.
4Approximately 10% of all newborns are admitted to the neonatal unit, where the neonatologists register data.
Measures

Handling of the biological samples
The blood samples taken from the mother (54 ml), father (4 ml) and child (10 ml) are stored at 4°C immediately after collection. The blood is then centrifuged at 4°C and separated into whole blood, plasma, serum and buffy coat, and distributed into cryo tubes (1.8 ml) before freezing (−80°C). The entire procedure for the parental blood samples, from blood sampling to freezing, is carried out within 2 h. The umbilical cord sample procedure is carried out within 24 h, during which the blood and tissue are kept at a maximum of 4°C. The time period from sampling to freezing is recorded in the database.

All participants and samples are registered in the biobank database, marked with barcodes and stored at −80°C. Each sample in the database has its own record with information on type of sample (e.g. full blood, buffy coat etc.), type of preparation (e.g. purified DNA, purified RNA etc.), and storage history (number of freeze-thaw cycles, alarms and temperature variations in sample protocols).

All biochemical results based on samples from the biobank are reported back and stored within the database. This means that the database will be a growing source of information as more and more researchers use the biobank material.

Specific measures in sub-cohorts
In order to purify RNA at a later stage in a sub-cohort, until December 2011 2.5 ml of blood from the mother were mixed in a vacuum tube with an RNA-stabilizer (PAXgene Blood RNA tube®) (6877 participants in total). The PAXgene® Blood RNA tube contains an additive that stabilizes the in vivo gene transcription profile by reducing in vitro RNA degradation and minimizing gene induction.8,9 This procedure was terminated in Dec 2011 due to lack of funding.

Analyses
With standardized treatment of the samples it is possible to study biomarkers with short half-life, for example certain cytokines and environmental exposures (e.g. diet-related). We will also be able to perform reliable toxicological measurements of various substances in fetal blood taken from the umbilical cord (e.g. xenobiotic chemicals such as perfluorinated chemicals and flame retardants, which are persistent compounds that do not degrade when kept at 4°C). Genetic studies and gene-environmental studies using the family trio design are also possible.10,11 Present day Next Generation Sequencing (NGS) requires more DNA than PCR-based methods, which has been taken into account when the dimensions for the biobank were set up (Table 1).

Box 1 Participation in the ABC Biobank

<table>
<thead>
<tr>
<th>Participation, total</th>
<th>45–48% a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete families (mother, father and infant)</td>
<td>62% b</td>
</tr>
<tr>
<td>Mother and infant</td>
<td>7% b</td>
</tr>
<tr>
<td>Mother only or mother and father only</td>
<td>30% b</td>
</tr>
<tr>
<td>Infant only</td>
<td>&lt;1% b</td>
</tr>
</tbody>
</table>

aPercentage of all women referred to give birth at Aarhus University Hospital, Skejby.  
bPercentage among Biobank participants.

Table 1 Overview of the sampling in the ABC Biobank

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Blood sample</th>
<th>Collection priority</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>Full blood (EDTA)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Mother</td>
<td>Plasma</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Buffy coat (EDTA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>Plasma</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Buffy coat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocyte suspension (Lithium-heparin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>Serum (clot activator)</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>Mother (sub-cohort: Aug 2008–Dec 2011)</td>
<td>RNA, mRNA, miRNA (PAXgene)</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Father</td>
<td>Full blood (EDTA)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Infant</td>
<td>Plasma</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Buffy coat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocyte suspension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td>Cord tissue</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
The PAXgene Blood RNA samples can be used to perform gene expression analysis and to measure the microRNA expression pattern. MicroRNA (miRNA) are small non-coding RNA sequences that regulate the translation of mRNA and work as biomarkers in several diseases such as cancer, rheumatoid arthritis and sepsis. It is also possible to do a metabolomic analysis of the plasma samples by nuclear magnetic resonance (NMR) to obtain metabolomics profiles in different groups of the cohort.

Validation of the quality of the biological samples

In the autumn of 2009, the Aarhus Birth Cohort Biobank and AROS Applied Biotechnology (http://www.arosab.com) validated the blood sampling methods by purifying DNA (cDNA, DNA) and RNA (mRNA, microRNA) from blood and umbilical cord tissue samples.

The procedure for collection of blood samples in PAXgene Blood RNA Tubes was tested. These tubes contain a stabilizing solution that protects RNA from degradation. However, in order to work properly, the tube must be filled with the correct volume and mixed immediately after phlebotomy; otherwise, coagulation may occur and no or only very little intact RNA can be extracted. We tested the procedures implemented by extracting RNA from 20 tubes. The average weight of the filled tubes was 19.18 g (range 18.99–19.27 g), which is well within the acceptable range. Extraction of RNA from the tubes was performed on a Biorobot MDx (Qiagen, Hilden) and resulted in an average yield of 8.62 μg (range 4.24–14.12 μg). Analysis of the RNA on an Agilent Bioanalyzer showed highly intact RNA to also contain small RNA species.

We also tested the extraction of DNA from the umbilical cord tissue samples. Extraction of pieces of 20–40 mg of tissue on a QIAsymphony SP instrument (Qiagen, Hilden) gave a mean yield of 2.21 μg (range 1.55–3.56 μg) of high molecular weight DNA. The umbilical cord tissue samples are in the range of 1–2 g, which would allow for the extraction of more than 60 μg of DNA from each sample.

Data resource use

In a recent study, blood samples from ABC Biobank were used to evaluate different methods for screening for trisomy 21 in early pregnancy. The study evaluated the diagnostic effect of measuring maternal serum markers twice during the first trimester. The study suggested a potential of the double maternal blood sample to improve the screening performance, and hence reduce the number of invasive prenatal examinations.

Strengths and weaknesses

The main strengths of this cohort are the high quality and the large volume of the biological samples. The few other Danish biobanks within our age category have limited volumes of biological material, and the material has not been processed and frozen within such a short time after sampling as have the blood samples in the ABC Biobank. The combined collection of biological material, questionnaires and clinical data (summarized in Figure 1) enables the monitoring of the consequences of time changes in environmental, health and dietary recommendations. Besides, the blood samples can be used to validate information from the questionnaires (e.g. information on medicine, intake of fish oil and vitamins). By being a part of an ongoing cohort, the number of samples will increase over the years and reach a size that permits studies of rare exposures or endpoints. Besides, most existing or planned cohorts are based on a fixed population recruited at a given time. ABC Biobank is an ongoing data collection and therefore new projects can be incorporated. The collection of biological material can be extended and adjusted to meet specific sample-taking and procedural requirements. For instance, we have currently extended the basic collection of biological samples to include 30 ml of serum (normal volume is 10 ml) in order to provide biological material for the FETOTOX project mentioned above. This project also includes a 3-year follow-up study in a sub-cohort. Another current addition to the basic data collection is the 10 ml EDTA (ethylene-diaminetetraacetic acid) stabilized full blood from the mothers, which is processed by specific methods in order to be able to purify and sequence DNA from the fetus. Another strength of the cohort is its location at the University Hospital, Aarhus. This hospital serves as the referral unit for the entire region for specific complications during pregnancy including specific fetal anomalies, e.g. cardiovascular, central nervous, urinary tract or genital malformations. Hence, the biobank provides a unique opportunity to study genetic and environmental causes of these diseases. Finally, Denmark is an ideal location for cohort studies, since we can follow the participants over time through childhood and adulthood by means of the Danish civil registration number, and we have easy access to clinical and demographic data (e.g. the Civil Registry System and the Patient Registries run by the Danish National Board of Health).

The main weakness of this cohort is the lack of capacity to recruit more than eight families per day on
average, and that we collect data from one hospital only. A simpler blood sample processing would allow us to extend the project to maternity wards in other hospitals. It is also a weakness that a small number of the biological samples from the umbilical cord are kept for up to 3 days before they are further processed (that is if the mother gives birth on a Friday afternoon).

**Data resource access**

The Biobank samples are available for researchers worldwide. To qualify for an extraction from the Biobank, an application including a research protocol should be submitted to the independent committee appointed by Aarhus University (for contact information: www.ab-biobank.dk). Interested researchers are expected to pay for material and working hours to extract samples from the Biobank, and a copy of all derived data should be returned to the Biobank. The ABC Biobank has a web page, which is regularly updated on number of participants, new projects, publications and funding.

**Funding**

This work is supported by the Danish Council for Independent Research, the Danish Council for Strategic Research, the A.P. Møller Foundation for the Advancement of Medical Science, TrygFonden and the Aarhus University Research Fund.

**Conflict of interest:** None declared.

---

**KEY MESSAGES**

- The Aarhus Birth Cohort Biobank is based on three main principles, as follows.
- High quality biological samples undergo quick processing in larger volumes than in most other existing biobanks.
- Blood sample collection from the mother, father and child allows for case-parent triad design.
- Ongoing data collection provides opportunities for specific sampling for new projects, evaluation of changes over time and combination of data from the biological samples with data from health registries, via the unique linking opportunities in Denmark.

**References**