Folate may reduce the incidence of autism

A growing number of health problems can be linked to environmental influences
A cohort study is a long-term effort. From the first concept to the start of MoBa it took five years. It took ten more years to reach the goal of 100,000 included pregnancies. Only when the children become adults will we get answers to the questions we posed at the start. We want to follow the children and their parents for years to come.
A lot has happened in the last 15 years, but our basic aim remains the same. We need more knowledge to enable us to prevent and treat serious diseases. Continued research is needed! Almost 300 scientific articles have been published in international journals using data from MoBa. We can use this as a measure of great interest from researchers both in Norway and worldwide. Are we any closer to preventing serious diseases? The answer is both yes and no. We have not solved any of the major mysteries yet. However, a number of theories and myths about links between environmental factors and disease can be discarded. They are incorrect and we do not need to introduce measures. We have removed the uncertainty.

We have also found many signs of potential causal relationships. When these results are published internationally, other research groups can take our findings and work on these issues using other samples and methods. We have big ambitions for cutting-edge research using data from MoBa in the years to come. We will continue to communicate our results as best we can to participants, decision-makers in government and business, and other researchers. We will also invite some of the participants to take part in the sub-projects, and we will send out more questionnaires. To solve more mysteries we need our participants to stay active. I hope that you and your children will stay on the journey!
FOLATE MAY PREVENT AUTISM

Ten years of Norwegian-American collaboration in identifying the risk factors for developing autism have borne fruit. The main finding is that folate supplements before and during pregnancy can reduce the incidence of autism by 39 per cent.

The ABC study (Autism Birth Cohort) is a collaboration between the Norwegian Institute of Public Health, Columbia University in the USA and the NiWaals Institute at Løvisenberg Hospital in Norway. The main funding – over $16 million – comes from the National Institutes of Health (NIH) in the USA. The purpose of the ABC study is to identify children with autism in MoBa, find causes of autism and study what happens to children living with autism.

When the project began in 2003 the researchers were very optimistic. They thought they would find MoBa children with autism and autistic-like conditions by means of the questionnaire that mothers completed when their child was 36 months. It turned out that it was not so easy, and they concluded that screening must be performed at several ages to find children with autism spectrum disorders (ASD). Most that were discovered by the age of three had such large deviations in language development and behaviour that they most likely would have been identified by the health service regardless of this screening.

Autism spectrum disorders (ASD) is a type of developmental disorder involving difficulties in reciprocal social interaction, communication and repetitive behaviour.

The symptoms vary from person to person. Some have severe developmental delay and very little speech, while others have good intellectual ability and normal speech. As the researchers did not find children with autism via the questionnaire at three years, they had to find another way. In 2008, the ABC study was the first in Norway to link data from the Norwegian Patient Registry to MoBa data to find children with ASD. This was a breakthrough. Without this ability to couple data from multiple registries, the ABC study would have been unsuccessful. Through the first link to the Norwegian Patient Registry, researchers found almost a hundred children with ASD in MoBa. They were invited to participate in the ABC study. After this, annual couplings have been performed and invitations to participate sent out.

MoBa researchers had a hypothesis that a lack of folate before and during pregnancy could lead to autism. British researchers had previously shown that folate deficiency can lead to a variety of developmental disorders called neural tube defects in the child. One of the most serious is spina bifida.

MoBa researchers showed that folate supplementation four weeks before conception and during the first eight weeks of pregnancy could reduce the number of children developing autism and Asperger syndrome with a startling 39 per cent. Researchers have thus strengthened the theory of the importance of folic acid for the repair and reconstruction of the cell's genetic material in the foetus.

The researchers have followed the development of folate intake in pregnant women participating in MoBa and have seen that usage has risen. 43 per cent took folate supplements in 2002, but six years later, 84 per cent of women said they had taken supplements in this important, early stage.

The goal, of course, is that all pregnant women – regardless of nationality – should get folate supplementation, either through fortified foods or a simple vitamin pill.

GPs and health centres are often the first to meet a child with suspected autism. The ABC study has shown that there is considerable variation between Norwegian counties in terms of diagnosis. Four to five times as many children have autism diagnosis in counties with the most diagnoses compared with those counties that have the fewest autism diagnoses. The researchers do not yet know what the reasons are for these differences.

FACTS

Autism spectrum disorder (ASD) is a type of developmental disorder involving difficulties in social interaction, communication and repetitive behaviour. The symptoms of ASD vary from person to person. Some have severe developmental delay and very little speech, while others have good intellectual ability and normal speech.
**Does it Matter What Mother Eats?**

Diet before and during pregnancy can affect the health of both mother and child. What the father eats may also be important.

**Mola has many sub-studies that have examined the relationship between the diet and health of nearly 90,000 mothers and their children. The researchers have studied the importance of diet for:**

- Birth weight
- Length and head circumference at birth
- The child’s size in relation to length of pregnancy, i.e., whether the child is large or small at birth
- Length of pregnancy
- Premature birth
- Pre-eclampsia
- Maternal weight gain and weight after birth

**The results support the official Norwegian recommendations that pregnant women should regularly eat vegetables, fruit, whole grain products, fish and dairy products, and drink water. They also show that it is beneficial to reduce the consumption of sugar, sweetened drinks, ready meals and salty snacks. Those who follow the advice reduce the risk of pregnancy complications such as premature birth, pre-eclampsia and low birth weight.**

*Other Mola studies have shown that:*  
- A mainly whole grain and vegetable diet can prevent pre-eclampsia  
- Mothers who follow the dietary recommendations are more likely to avoid obesity in the months after birth  
- The advice to avoid eating foods that are known to contain environmental contaminants is sound  
- There is a positive correlation between the consumption of lean fish and fetal growth  
- It is good to consume so-called probiotic dairy products (Bifidobacteria etc.). This appears to give a good intestinal flora which strengthens the body’s immune system  
- Pregnant women who eat organic vegetables have a lower risk of pre-eclampsia.

**Researchers did not see this association if pregnant women ate organic fruit, cereal, eggs, milk or adhered to a predominantly organic diet. Researchers believe one possible reason for reduced risk when consuming organic vegetables is that organically grown vegetables contain fewer pesticides.**

Diet before and during pregnancy can affect the health of both mother and child. What the father eats may also be important.
WHEN WORDS GET STUCK

It is common for some children to take longer than others to develop their language skills. Some never catch up and have long-term language difficulties. MoBa researchers are trying to figure out why.

It begins with imitation. An infant follows their parents’ sounds and movements. After a while, the child begins to imitate, and sounds become words. The child has been given a tool for learning, coping and bonding with other people.

For seven to ten per cent of children, words do not come easily. Some also struggle to understand what others say. When these children grow older, they lack the tools used for homework and playtime. Language difficulties can often lead to other problems.

The Language and Learning Study (SOL study) aims to find out what contributes to good language development, the early signs of language problems and why some children develop language difficulties.

Most children begin to utter their first words at around one year old. Over the next year, many parents realise that children understand much of what they say, and learn new words daily. By the age of two, children can make two-word phrases. By the age of three, most children will have developed quite a lot of speech. The SOL study started in 2007 and is a collaboration between the Norwegian Institute of Public Health, the Ministry of Education, schools and childcare centres. The goal is to provide the best possible knowledge base for understanding the causes and pathways of language difficulties, so that preventive measures can be introduced at an early stage.

MoBa has given teachers and parents a unique opportunity to do just this. Using questionnaires that the mothers filled out when the child was 6, 18 and 36 months old, researchers have information about children at an important language development period. By eight years old, some of them may have been diagnosed with specific language impairments. Some will be invited to participate in a clinical study (Språk-8) in which their scores are compared to children without specific language difficulties in a variety of language tests. This allows researchers to compare children with language disorders with those with normal language development, and to see if there are common characteristics that distinguish them earlier in life. They hope this will provide clues to what causes language difficulties.

Studies abroad have concluded that boys are more prone to develop language difficulties than girls. The SOL study confirms that this is the case in Norway. Both in the group of “persistent” and “transient” language difficulties, boys are in the majority.

The SOL study investigated how childcare affects the development of a child’s language and learning abilities. Researchers found that children who have good relationships with adults in the childcare centre do better linguistically and psychologically than children with poor relationships. Other aspects of childcare, such as educational practice, material resources, number of staff and group size had little effect.

As the children are part of the MoBa study, the researchers will be able to follow them. These data will be used to find out how language disorders affect children when they get older.
FROM QUESTIONNAIRE TO RESEARCH

So far, MoBa has received about 800,000 questionnaires. Making an analysis file from the questionnaires for use in research is a time-consuming and complex process. To keep track of the progress of the questionnaires, an administrative database system (MoBaStudy) has been developed. Participants are registered with their name and address and when the questionnaires were sent out and returned. All biological samples that are donated by participants are also registered. In another database (MoBaData), the answers from each questionnaire are stored. These two databases are completely separate.

3. SCANNING
All questionnaires are designed so that they can be scanned by a computer program. On each page of the questionnaire there are five crosses which the scanner uses to recognise the form. If something has been ticked off or written that doesn’t make sense, for example, a date that does not exist, the computer program will capture this.

4. INTERPRETATION AND QUALITY ASSURANCE
When the computer program interprets the data, any abnormal values are marked in yellow. These values are checked against what the participant marked in the questionnaire. This control is called verification. The verified data can then be loaded into the MoBa database.

5. CODING
Medicines, diseases and occupations are coded according to international, standardised codes.

6. BIOLOGICAL SAMPLES
During pregnancy and at birth, blood samples were taken from mother, father and the child’s umbilical cord. These blood samples are stored in the MoBa biobank (see article page 16). Blood samples are sent for analysis and the laboratory results are returned to MoBa.

9. ANALYSIS FILE
When all the data from the questionnaires, any additional investigation studies, and analysis results from blood samples are quality assured and coded, an anonymised data analysis file is available to researchers. Since each participant is given a random number, their questionnaire data can be followed over the years, but information that could identify participants is never released to researchers.

8. DATA FROM OTHER REGISTRIES
For more information about disease and health, MoBa needs to obtain information from other health registries. Before MoBa can link registry data and study data, the Regional Committees for Medical and Health Research Ethics and sometimes the Norwegian Data Protection Authority must grant permission for the research project.

7. ADDITIONAL STUDIES
Participants may be asked to participate in additional studies where we need more detailed information about individual conditions or health problems. Additional studies must be specifically approved by the Regional Committee for Medical and Health Research Ethics. In addition, MoBa participants who agree to take part must complete a consent form for each study. The ABC Study is an example of an additional study (see page 6).
MANY QUESTIONS BUT FEW ANSWERS

Researchers hope that blood from the mother, father and child can provide new knowledge about childhood epilepsy.

The idea came from Scotland. Professor Richard Chin at the University of Edinburgh wanted to use MoBa data to study the incidence, cause and risk factors, and development of children with epilepsy. In 2013, the EPYC project (Epilepsy in Young Children) became a reality with funding from the Research Council of Norway.

Childhood epilepsy is one of the most common serious neurological disorders in children and we estimate that the incidence is between 0.5 and 1 per cent. This means that between 300 and 600 Norwegian children are born every year who will receive this diagnosis.

Research has shown that the incidence in recent years has fallen in countries with high living standards. Unfortunately, this field of research is small and there is still a lot we do not know about epilepsy in children.

Some cases of childhood epilepsy have known causes, such as intracranial bleeding, infections and other brain damage before, during or after birth. Of the other cases, 30 per cent had identifiable causes, usually genetic mutations. However, the remaining 70 per cent had an unknown cause. The researchers believe that many of these also have a genetic cause, but not enough is known about which genes are involved.

EPYC aims to contribute new knowledge about the causes and risk factors in childhood epilepsy that currently has an unknown cause.

MoBa researchers can use blood from the mother, father and child to study genetic factors. This provides unique opportunities to study heredity and identify genes that may be involved in disease development.

There are no other population studies of the scale of MoBa that have the blood from the mother, father and children so MoBa can really make a difference. By studying the blood of parents and children, researchers could uncover new knowledge with potential to help with prevention, and perhaps new treatment methods for childhood epilepsy.

In the future, MoBa researchers plan to study the epigenetic factors that may be involved in the development of epilepsy. No researchers have done this before.

Researchers now know that it is not only changes in the genes of the child – inherited gene mutations and spontaneous mutations – that can lead to developmental disorders.

Genes can also be switched on and off during critical periods of fetal development by external factors. These epigenetic changes may damage the fetus and lay the foundation for disease later in life.

The researchers will study risk factors such as maternal diet, lack of folate intake, smoking during pregnancy, high blood pressure and stress in the mother, parental age and complications during pregnancy and childbirth, as well as the child’s birth weight.

840 MoBa children have been diagnosed with epilepsy and 650 are in the EPYC study.
MILLIONS IN THE BANK

The biobank contains biological material donated by participants in health studies organised by the Norwegian Institute of Public Health. These samples will provide invaluable knowledge about health and diseases.

The biobank was established in 1999 when the Norwegian Mother and Child Cohort Study (MoBa) began. Responsibility for the biobank was given to the Norwegian Institute of Public Health when the institute was established in 2002.

The biobank has an area of around 2,000 square metres for storage of biological samples in various units. We have many chest freezers in the storage areas, but the majority of samples are kept in two large automated freezers that are maintained at minus 20 and minus 80 degrees. Both freezers can store several millions of samples at minus 20 and minus 80 degrees. Both freezers can store several millions of samples at minus 20 and minus 80 degrees. Both freezers can store several millions of samples at minus 20 and minus 80 degrees.

Distribution of biological material
• Samples are thawed and the required amount is sent to researchers whose applications to use the biological material have been approved.

For most studies, the biobank contains many aliquots per participant. In total, there are several million aliquots from MoBa alone. We have four million aliquots of DNA.

Researchers can apply to receive biological samples from the biobank for use in research projects. All applications are considered by steering groups at the institute who will grant access to data according to set guidelines.

In theory, with the correct storage conditions, biological material can be stored for a hundred years. However, more research is needed to know more about, for example, the condition of plasma and urine after several years of storage. The biobank carries out regular quality control and requests feedback from the researchers using material from the biobank.

The biobank has its own quality system, and was recently ISO 9001 certified. The biobank follows internal procedures to ensure that quality and safety are maintained. We have rigorous training programs, a nonconformity handling system, and a continuous improvement focus. We follow best biobanking practice, for example how long the different samples can be stored at room temperature.

The way ahead
The biobank has several goals for the future and is working to:
• Become more efficient, take advantage of the investments made in fully automated freezers.

Build an organisation that can handle multiple external projects, taking advantage of expertise, facilities and equipment in the biobank today.

Develop national biobank services, through Biobank Norway and regional biobank collaboration.

Develop an infrastructure for the best methods to extract and analyse samples from teeth.

Develop a nonconformity handling system, and a continuous improvement focus.

We follow best biobanking practice, for example how long the different samples can be stored at room temperature.

MILK TEETH

Milk teeth are a kind of black box recorders that give researchers important information about health and disease. The MoBa Tooth Bank in Bergen (MoBa Tann) now contains milk teeth from more than 24,000 children in the MoBa study.

The tooth bank contains milk teeth from children taking part in the MoBa study. They are encouraged to submit one or more shed milk teeth. MoBa sends out information about this shortly before the child reaches seven years of age.

Milk teeth are usually shed early in life and are a kind of data recorder that give researchers important information about health and causes of diseases. Teeth store information about environmental factors that the child has been exposed to since they were in the womb.

Teeth can tell what the mother ate during pregnancy and about uptake of contaminants, such as lead and cadmium. These substances can affect the child in the womb and in early childhood. Along with MoBa information from questionnaires and blood tests, results from tooth analysis can tell researchers about the impact that hazardous substances have on children’s health.

Teeth are formed in layers, and we can see the difference between the part of the tooth that is formed before birth and the part that is formed afterwards. Dental tissue is the only stable tissue formed in the fetus and in infancy that is available for analysis on a large scale at a later date. In the future, information gained from the teeth could help to prevent disease or other conditions.

Milk teeth have an almost unlimited shelf life and can therefore be useful to researchers for many years to come. On arrival, the teeth are registered and anonymised and stored in secure storage boxes at the University of Bergen. Researchers are now working to find the best methods to extract and analyse samples from teeth. New analytical methods can increase the value of dental material even further.

It is also a goal to establish standards for how analyses should be performed so that researchers can compare findings in teeth from different countries. MoBa Tann researchers are working with researchers in Canada, USA, UK and Germany.

MoBa Tann is a collaborative project between the University of Bergen and the Norwegian Institute of Public Health.

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24 000
CHILDREN IN THE MOBA STUDY HAVE SENT US ONE OR MORE MILK TEETH.
Antenatal and maternity care is improving in Norway. Even so, the percentage of children born with cerebral palsy is not falling. Norwegian and Danish researchers have teamed up to find out why.

Previously, it was believed that most cases of cerebral palsy (CP) occurred during birth. Now, researchers reckon that only ten per cent of cases are due to birth injury, and that we should be looking at what happens to the fetus early in development.

Although CP is the most common cause of physical disability in Norway, the condition is rare. Only two out of every 1,000 children is diagnosed with CP. There is relatively little research into CP. Therefore, researchers in Norway decided to combine their efforts with colleagues from the Danish National Birth Cohort (DNBC) to study the children with CP in the Norwegian and Danish cohorts. A working group from Bergen and Copenhagen has merged relevant data from both studies to create a common Norwegian-Danish merged data set.

A group from Bergen and Copenhagen has merged relevant data from both studies to create a common Norwegian-Danish merged data set. The researchers identified 437 children with CP in MOBAND, of which 246 are from the Danish National Birth Cohort (DNBC) to study the children with CP in the Norwegian and Danish cohorts. The harmonised data in their work. Over 1,000 children is diagnosed with CP.

Children with cerebral palsy seems to have increased over the last 20–30 years. The reasons for this are still not clearly established and researchers want to know if genetic and environmental risk factors are involved. MoBa has an active research group that is looking at the interaction of various risk factors in pregnancy and the development of respiratory problems in children. The research group is using questionnaires and biological material from MoBa, plus data from the national health registries. In addition, researchers are collecting new data from children aged 10–11 years.

MoBa researchers have uncovered new knowledge about the relationship between smoking during pregnancy, maternal body mass index, intake of vitamins, including vitamin D and folate in pregnancy, and the development of respiratory problems in children. Folate intake by pregnant women in Norway has doubled since 1991, when MoBa researchers found that folate supplements could halve the risk of neural tube defects such as spina bifida in the infant. Recently, MoBa researchers have shown that folate intake can also significantly reduce the risk of developing cerebral palsy.

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ENVIRONMENTAL CONTAMINANTS – A GROWING PROBLEM

Every day, we are exposed to a large number of natural and man-made chemicals. In several MoBa studies, researchers have investigated how these unwanted substances in the environment affect health. In addition, 3,000 MoBa participants will be invited to become regular eco-monitors in a new environmental biobank project.

Environmental contaminants, found in the food we eat, the air we breathe, and the products we apply to our skin, can affect our health. Exposure to noise and UV radiation also affects us. Everyone is exposed, but infants and children are particularly vulnerable to potentially negative effects of environmental contaminants.

We are beginning to understand how individual factors in the environment can affect children’s health, but we know too little about how they interact in the so-called “cocktail effect”. With better knowledge it becomes possible to develop preventive measures to create a healthy and safe environment for the next generations.

Creating a human environmental biobank is part of the Norwegian Institute of Public Health’s strategy for the coming years. Using MoBa data, we will study some of the most vulnerable groups, including pregnant women, fetuses, and young children.

We will therefore invite 3,000 MoBa participants to become regular contributors to the environmental biobank.

The environmental biobank will be suited to study time trends in public exposure to environmental contaminants, and evaluate the effects of measures made to decrease exposure. We will also be able to check older samples for contaminants that have not yet been identified. Furthermore, the project will enable studies of how diet may affect how the body handles toxins. For example, people with low iron levels are apparently more likely to absorb heavy metals such as cadmium and lead.

Part of the research project will examine whether dietary factors protect against the harmful effects of toxins.

In a separate project ongoing in 2014/2015, the Norwegian Institute of Public Health has invited 300 MoBa participants living in the Oslo area to participate in the HELIX Child and Environment project, which is part of an EU-funded HELIX research project (The Human Early-Life Exposome). The HELIX project will also be studying the “cocktail effect”. We will study the environment around children, including air quality, noise, access to nature and parks, food and drink, and their physical activity.

Further, we will measure various environmental factors in the blood and urine and examine how genetic material can be affected by exposure. The relationship between environmental impact and various health indicators such as obesity, lung function and the child’s neurological development will also be studied.

As we have already gathered a lot of information about the MoBa children, researchers can learn a lot from this study.
Pregnant women were invited to take part in the Norwegian Mother and Child Cohort Study when they attended their ultrasound scan at weeks 17–20 of pregnancy. If they agreed to participate, the child’s father was also invited. During pregnancy, mothers completed three questionnaires and the fathers completed one. Each child’s development is being followed using questionnaires at six months, 18 months and three, five, seven and eight years. We want to continue to follow their progress and will send more questionnaires to the children and their parents as they grow older.