

A biobank of primary teeth within the Norwegian Mother and Child Cohort Study (MoBa): a resource for the future

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Summary

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Tvinnereim HM, Lygre GB, Haug K, Schreuder P, Klock K. A biobank of primary teeth within the Norwegian Mother and Child Cohort Study (MoBa): a resource for the future. *Paediatric and Perinatal Epidemiology* 2012; **26**: 264–271.

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based cohort study including >100 000 pregnancies and following the children through childhood, using questionnaires and collecting biological samples. The aim of MoBa is to test specific aetiological hypotheses by estimating the association between exposure and disease, aiming at prevention. A biobank for exfoliated primary teeth collected from the children participating in MoBa has been established (MoBaTooth Biobank). Samples of tooth tissues from the primary dentition can give information about exposure to toxic and essential elements during fetal life and early infancy. Prenatally and postnatally formed tooth tissues permanently document early exposures unlike other biomarkers, as teeth form incrementally at a known rate. Results from tooth analyses will be coupled with corresponding information in the multiple questionnaires and data from analysis of other biological samples collected by MoBa. Invitations to donate one or more teeth are sent to all mothers/children in the period 2008–2016, when the child is 6.75 years old. By August 2011, 7400 participants had been recorded into the MoBaTooth database donating altogether 9798 teeth (1.3 teeth per child). The response rate was 24%, but there is a lag of >1 year in the response. Data from the tooth biobank can supply MoBa with important additional information on the uptake of trace elements during fetal life and early infancy. This information can illuminate possible causal factors of health and disease in the future.

Keywords: MoBa, biomarker, biobank, teeth.

Introduction

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health.¹ Participants were recruited from all over Norway from 1999 to 2008, and 38% of invited women consented to participate. The cohort now includes 108 000 children, 90 700 mothers and 71 500 fathers. Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth² (Figure 1). Follow-up is conducted by questionnaires at regular intervals and by linkage to national registries. Several sub-studies

are collecting additional data and biological materials. The aim of MoBa is to test specific aetiological hypotheses by estimating the association between exposure and disease, aiming at prevention. The study is approved by all relevant Norwegian authorities, and follow-up is open-ended.

A biobank of shed primary teeth collected from the children participating in MoBa was established in 2008 to supply MoBa with additional biological samples. Human primary teeth have been used as biomarkers of exposure to a number of trace elements, toxic as well as essential,^{3–8} and relationship between exposures and health outcomes have been reported for several decades.^{9–11} Mineralisation of the tooth buds of

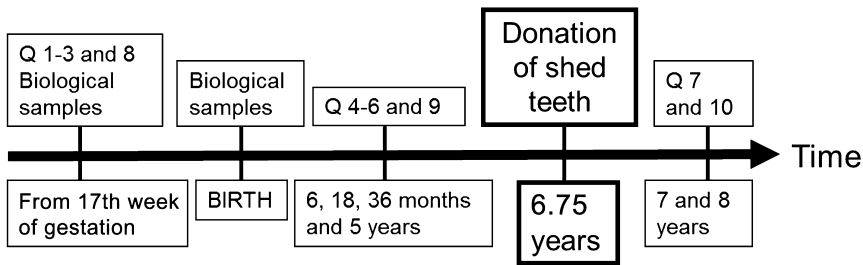


Figure 1. Time line for the collection of variables: questionnaires (Q 1–10), biological samples to the biobank of Norwegian Mother and Child Cohort Study (MoBa) and shed primary teeth to the MoBaTooth Biobank.

primary teeth starts at about the fourth month of fetal life and continues throughout the first years of childhood.¹² Trace elements, especially those which are bone-seeking, are incorporated into the tooth substance during tooth formation.¹³ In contrast to mineralised bone tissues, mineralised tooth tissues are very stable; once formed they do not remodel.¹⁴ Unlike other biomarkers, prenatally and postnatally formed tooth tissues permanently document early exposures, as teeth form incrementally at a known rate.¹⁵ This allows for the tracking of elemental fluctuations across months, weeks and even days.¹⁶ Thus, tooth tissues from primary teeth can give information about environmental exposure and nutrition during the most vulnerable time period in life: fetal life and early infancy.^{17–19}

Studies of trace element concentrations in dental tissues have traditionally used a bulk sampling approach or have relied on physical separation of different parts of the tooth prior to analysis. A common method has been to acid-digest the samples before analysis using atomic absorption spectroscopy (AAS).⁶ During the last decades, we have seen technical as well as methodological improvements in the analysis of mineralised tissues. Using laser ablation inductively coupled mass spectrometry (LA-ICP-MS), it is now possible to map trace element concentrations in tooth tissues to a high precision across the growth lines of ground sections of the tooth.^{20,21} The growth line mineralised around birth (the neonatal line) may be identified under the microscope and tissue samples formed before and after birth may be distinguished (Figure 2). The techniques for the analysis of calcified tissues are still being developed, and for several trace elements there is still a lack of appropriate solid reference materials that are matrix-matched for calibration purposes.²² It is likely that these limitations will be overcome in the future.

The overall aim of the tooth biobank project (MoBa-Tooth) is to study the effects of environmental and dietary factors on the health of children in MoBa by combining information from tooth analyses with the

comprehensive information available in the questionnaires and other biological samples collected within MoBa (Figure 3). MoBaTooth will provide a unique opportunity to trace sources of several elements and produce information to estimate associations between exposure and disease. The tooth collection will also

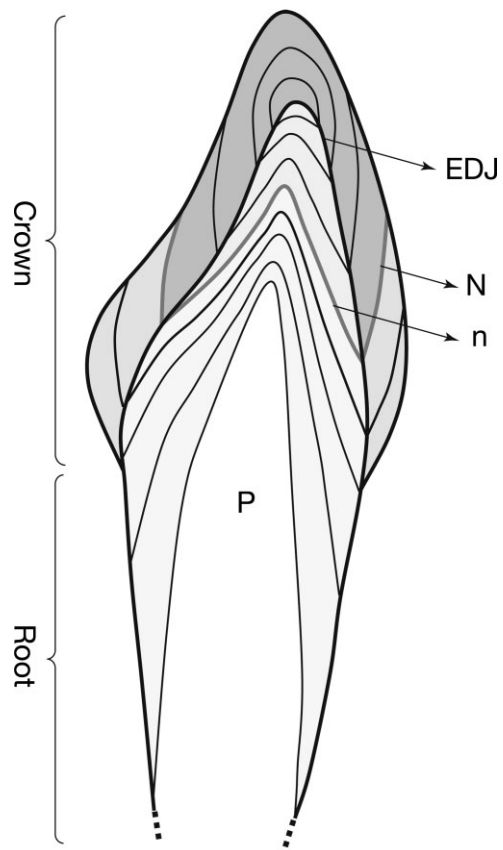
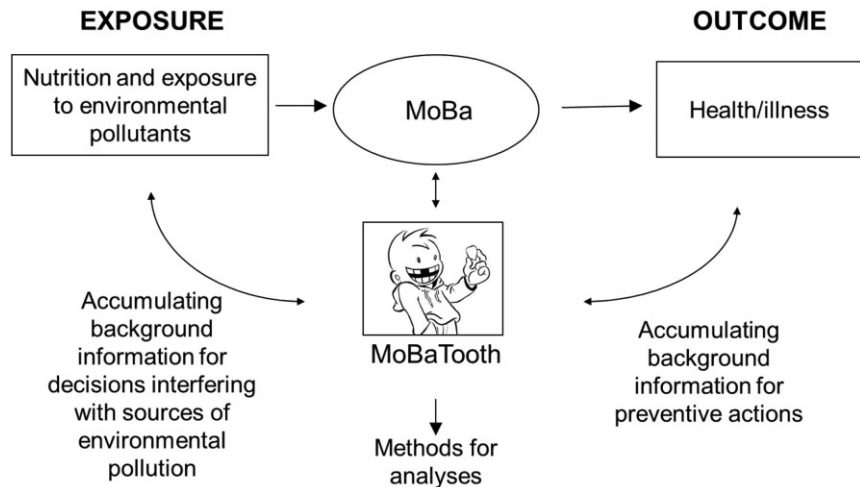


Figure 2. Illustration of a longitudinal sectioned primary incisor in development, showing growth lines in enamel and dentine [outer and inner part of the crown respectively separated by the enamel-dentine junction (EDJ)]. Note the separation of prenatally and postnatally formed enamel and dentine by the neonatal line (N, neonatal line in enamel; n, neonatal line in dentine). The prenatally formed enamel and dentine has the darkest colour. P, pulp chamber. [Correction added on 22 February 2012, after first online publication: Figure 2 was replaced with a new figure showing the proper labels for the illustration mentioned in the figure caption.]

Figure 3. Schematic presentation of information available within Norwegian Mother and Child Cohort Study (MoBa) and MoBaTooth to investigate associations between exposure/nutrition and health outcome.



provide a unique opportunity for further development of primary teeth as biomarkers of environmental exposure, nutrition and disease. In this paper, we present the methods used for sampling of primary teeth for the biobank, including recording, storage and retrieval.

Methods

The study population

The target population is all the 108 000 children participating in MoBa. The study is described in detail elsewhere.¹ The size of the study is based on the fact that many diseases and types of exposures are relatively rare (MoBa protocol: <http://www.fhi.no/morogbarn>). Children participating in MoBa are asked to supply one or more shed primary teeth at the age of 6.75 years. In 2006 the first children reached this age and by 2016 the last set of children will reach this age (Table 1).

Collecting teeth

A letter describing the MoBaTooth project is sent to all mothers participating in MoBa 3 months before the child reaches the age of 7 years. Participants are asked to donate one or more shed primary teeth and return

them in a polypropylene tube supplied to them with a return envelope. The information letter gives a brief description of the purpose of the collection and how parents should treat the tooth/teeth before postage. The teeth should be washed in clean water, without use of detergents, and left to dry overnight. The tooth/teeth should then be placed in the tube and put in a small envelope with the child’s unique study ID number for identification. The envelope is returned together with a statement of informed consent, signed by the parents. If the child has not yet shed any teeth, or has not kept them when shed, parents are encouraged to send the next shed tooth in the attached packaging.

Recording of data

The teeth are recorded in the MoBa database before being registered and stored in the biobank of MoBaTooth. The teeth are recorded in an authenticated and encrypted system developed in house. This system enables linkage between results from the analysis of each tooth to corresponding information in the MoBa database.

If a tooth has visible traces of blood, is still humid and/or smells, it is rinsed in distilled water and dried overnight before examination and recording of vari-

Table 1. Number of child participants (*n*) within Norwegian Mother and Child Cohort Study (MoBa) reaching the age of 6.75 years during the years 2006 to 2016

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
<i>n</i>	495	2 382	5 325	10 280	13 052	14 065	16 874	17 169	15 889	11 737	1 327

ables. All teeth collected are handled as possible infectious material. Gloves or tweezers are therefore used routinely when inspecting the teeth.

Variables

Tooth variables include tooth type (upper and lower central and lateral incisor), caries (scores 0–2), attrition/abrasion of the incisal edge of the tooth crown (scores 1–3) and root resorption (scores 0–4) (Figure 4). Internal discolouration of the tooth crown, and enamel hypoplasia/hypomineralisation are also recorded when present (scores 0–1). It is possible to record free text such as information on excessive calculus, external discolouration and extremely abraded tooth crown. Variables are recorded by calibrated personnel using a Luxo magnifier lamp (Bürklin, Oberhaching, Germany). A manual has been developed for the purpose and a test is performed to evaluate the quality of the recording work.²³

The biobank

Handling and storage of the teeth are done in accordance with the Norwegian Biobank Law (<http://www.lovdata.no/all/hl-20030221-012.html>, accessed January 2012). Teeth have an almost unlimited shelf life when kept dry at normal room temperature.²⁴ The teeth are stored in the original package they were received in. Access to the tooth material is given according to current policies in MoBa (<http://www.fhi.no/>).

Ethical and legal issues

The MoBaTooth project has been approved by all relevant institutions in Norway. The database of the tooth biobank gives no access to information about the identity of donors. The participants are informed that they will not receive individual results from tooth analyses. Future findings may be presented on

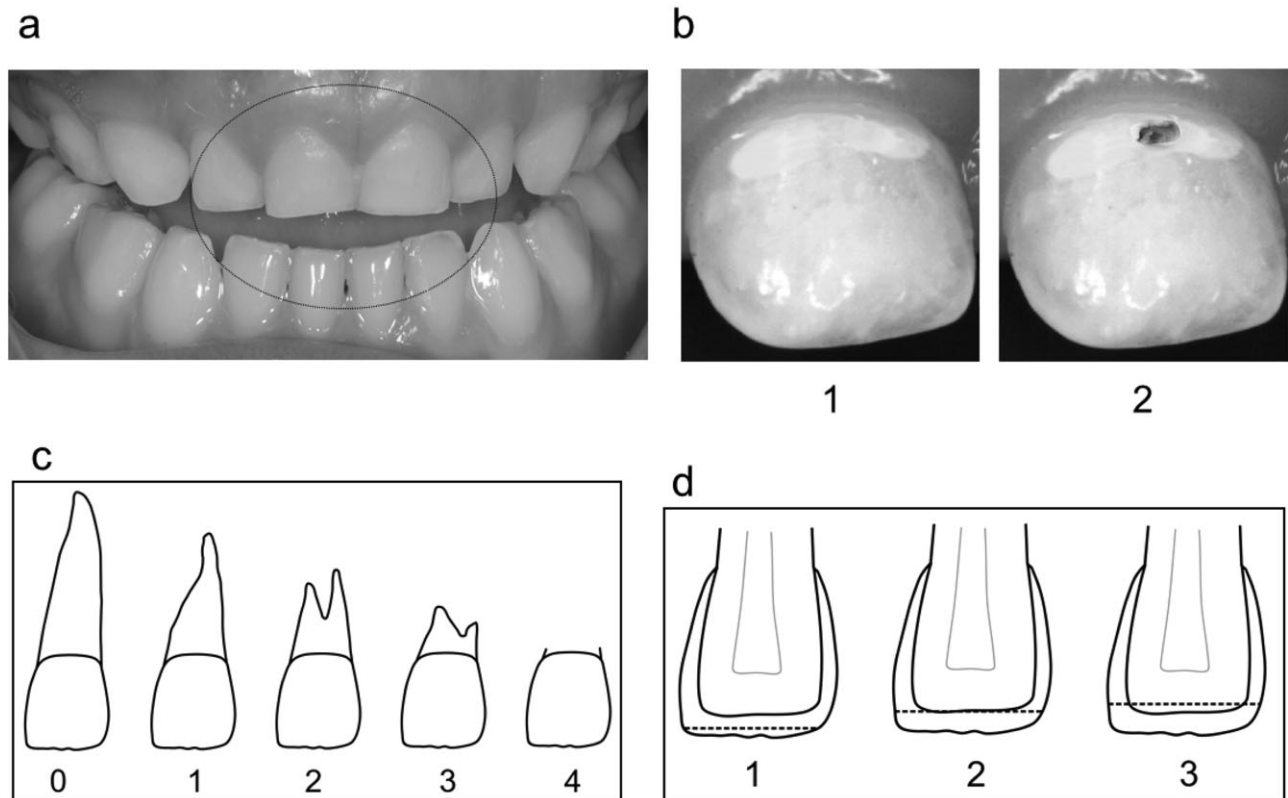
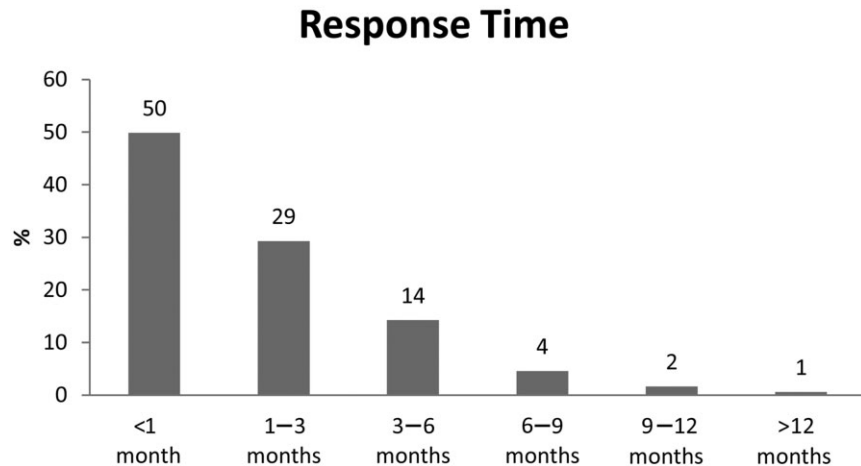


Figure 4. Variables recorded in the database. (a) Primary dentition with the actual tooth types encircled. (b) Examples of degree of caries (1 = enamel caries; 2 = caries with cavitation to dentine*). (c) Degree of root resorption (0–4). (d) Degree of attrition/abrasion of incisal edge of the tooth crown (1 = only in enamel, 2 = to the enamel–dentin junction, 3 = further into the dentine). *Courtesy of Prof. Ivar Espelid and *Tandläkartidningen* (Espelid I, Tveit AB, Mejäre I, Nyvad B. Karies – ny viten eller gamle sannheter? *Tandläkartidningen* 1997; 89:19–28).

Figure 5. Distribution of respondents in per cent according to the time period from receiving the invitation to participate with teeth in the MoBaTooth project until the response letter with teeth was received by Norwegian Mother and Child Cohort Study (MoBa).



the web page of the Norwegian Institute of Public Health and in the yearly newsletter sent to the participants of MoBa. It is specified that the mother/child can withdraw at any time from participation in the MoBaTooth project and requires the teeth be destroyed and the data deleted. For each research project intending to use tooth material from the biobank and data/biological samples from MoBa, an application has to be sent to the current regional ethical committee (REK) in Norway for approval of the actual project (<http://helseforskning.etikkom.no>).

Results

Status to date

A total of 9798 primary teeth, representing 7400 children, had been registered into the MoBaTooth database by August 2011. The response rate was about 24% of the participants that had received an invitation to donate teeth by this point of time. The number of teeth offered per donor varied from one to seven teeth with a mean of 1.3 per child. There was no difference in gender distribution. The response time varied from a few days to >1 year (Figure 5).

Altogether, 45% were upper front teeth, 52% were lower front teeth and 3% were classified as uncertain tooth type. Carious lesions were found in 1.5% of the teeth, 85% had >2/3 of the root resorbed, 63% of the teeth were markedly worn at the incisal edge, 1.9% had internal discoloration and 0.8% of the teeth had obvious areas of hypoplasia/hypomineralisation on the enamel surface.

The majority of the teeth were clean and dry. Only a small number showed traces of blood or other soft organic residuals on the surface or were humid or bad-smelling. Calculus at the surface was frequently seen.

Discussion

The MoBa is a cohort study where the individual is recruited to the study prior to the onset of the disease/dysfunction that is to be studied. Linkage to other Norwegian health registries (e.g. Medical Birth Registry or Cancer Registry) or exposure registry (National census data) will enable new data sets to be generated simply and effectively.¹

The primary teeth collected will constitute a biobank in the MoBa system, and the information from analyses of variables will supplement the vast amount of information in the main study. Many of the subprojects will be based on the cohort design of the main project. When teeth samples are being analysed, the relevant design will be a nested case-control study. This involves identifying a sample of subjects that have developed the disease or dysfunction to be studied and selecting controls that have not developed the disease. Trace element concentrations will be measured in teeth for both groups to look for differences.

Selection to MoBa

Selection of participants for the main study is described in the MoBa protocol (<http://www.fhi.no/morogbarn>). The population was all pregnant women. The most important bias is a lack of willingness to

participate. A participation rate of about 40% is acceptable since the main purpose is to calculate the associations between exposure and illness. MoBa is not designed for studies of incidence and prevalence. The incidence of caries of 1.5% found within the tooth collection may thus not be representative of the Norwegian child population, as the incidence of caries varies for instance by the parents' educational level.²⁵

Selection to MoBaTooth

The relatively low response rate (24%) may indicate that some of the children had not shed teeth by the time the parents received the letter from the MoBa-Tooth project, or they may have lost them or thrown them away. The response rate may increase during the collection period as there is a delay in response. Approximately half of the donors responded within the first month while some responded after 1 year (Figure 5). Many of the children/mothers invited might prefer to keep the teeth as valuable mementos from childhood.

Why ask for teeth at about 7 years of age?

We decided to collect primary front teeth which are the first teeth to be developed and shed. More than two-thirds of the crown of front teeth is developed before birth. The crown therefore consists of a large bulk of prenatally formed tooth substance. Furthermore, the front teeth are less often attacked by caries than the molars. The front teeth are commonly shed in the period between 5 and 9 years, the central incisors first, followed by the laterals.²⁶ Collecting teeth from the MoBa children at the age of 6.75 years was chosen for practical reasons.

In the invitation letter, we ask for shed primary teeth without specifying a certain tooth type. This was done to make it easier for the participants. On the other hand, it would have been an advantage to collect the same tooth type from all the participants. The central incisors of the upper jaw would be the preferred ones, as these teeth are the largest that are shed at that age, thus providing more material for the tooth analyses. So far, 30% of the teeth collected are central incisors from the upper jaw.

Handling of the teeth

The parents are asked to wash the teeth in clean water without detergents. The teeth are not treated further

before storage, thus avoiding the introduction of any chemicals or high temperature regime that might interfere with future study methods. The tooth material must therefore be treated as potentially infectious.

Registering of tooth variables into the database

The variables selected to be recorded into the database for each tooth were primarily information describing available material in the specific tooth. A non-carious tooth contains more tooth substance representing the tooth formation period than a carious tooth, as carious lesions are demineralised and may exchange elements and substances with the oral cavity.²⁷ Incisal wear of the tooth crown gives information on the degree of loss of the earliest formed tooth substance in the incisal region, and the degree of root resorption gives an estimate of root substance available.

The infrequent incidence of internal discolouration indicates former bleeding in the pulp chamber, as traces of blood have been integrated into the peripulpal dentine, giving rise to elements not originating from the period of tooth formation. Thus, discoloured teeth are not preferred.

Storing of the teeth

In determining the logistics of the tooth biobank, issues of optimal storage of the teeth were balanced by the costs. It was decided to store the teeth in dry conditions at room temperature. Mineralised tooth tissues can be stored indefinitely in this way. However, storing the teeth in freezers may prevent the teeth from drying out and break into pieces. Furthermore, freezing of the teeth may prevent the disintegration of persistent organic pollutants that might have been integrated into the organic parts of the tooth tissues during tooth development.²⁸ In the 'Avon Longitudinal Study of Parents and Children (ALSPAC)',²⁹ primary teeth have been stored in freezers in the same way as the other biological samples collected (personal information from Amanda Carmichael, Deputy Director, ALSPAC). At present, analytical methods for measuring persistent organic pollutants in mineralised tooth tissues are not available as far as we know, but may appear in future research.

Future perspectives

Tooth tissues are the most chemically stable tissues in the body and can be stored indefinitely at room tem-

perature when kept dry. The collected teeth will be stored and made available to scientists in the future. The likely development of new and improved methods will offer the opportunity to investigate new hypotheses of possible associations between exposures and outcomes. These teeth may provide information about causes of diseases and malfunctions in childhood, but may also contain information that will be of great value in the future, when the children grow into adulthood. Findings of associations between exposure and disease are of importance in developing preventive strategies. Furthermore, findings pointing to sources of pollution can lead to political action aimed at preventing such problems. As a start, we plan to randomly allocate teeth from 300 donors within the MoBaTooth Biobank to a mapping of trace element concentrations in the population to give a platform for the development of study approaches.

Conclusion

The biobank of primary teeth will supply MoBa with important additional information on the uptake of trace elements and substances during fetal life and infancy. This information can illuminate causal factors of health and disease in the future.

Acknowledgements

The donation of teeth, other biological material and questionnaire data from MoBa participants are gratefully acknowledged. The MoBaTooth project has been supported by grants from the University of Bergen through funding from Department of Clinical Dentistry, the Norwegian Research Funding, the Meltzer University Fund and the Norwegian Dental Depot Fund. Thanks to Professors Rune Eide and Nils Roar Gjerdet, Department of Clinical Dentistry – Biomaterials, UoB, for their support during the establishment period of the biobank, data engineer Ole Martin Kvinge and adviser Marianne Bjørnen at the Norwegian Institute of Public Health for developing the database for MoBaTooth and administering the practical part of the collection of the teeth, respectively; and to engineer Odd Johan Lundberg and research technician Randi Sundfjord at Department of Clinical Dentistry assisting in the administration of the biobank.

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