Chapter 10
Current Findings in a Birth Cohort Study with Omics Analysis: Chiba Study of Mother and Child Health (C-MACH)

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Abstract Recent epidemiological studies have shown that environmental factors like maternal nutrition, smoking habits, and economic stability during the period from pregnancy to early childhood might affect the risk of noncommunicable diseases in adulthood. This concept is referred to as "developmental origins of health and disease (DOHaD)." Exposure to chemicals is also an important risk factor for fetal development. Moreover, there is some concern that certain chemicals can affect human reproduction and development because of their endocrine-disrupting properties, especially during fetal period.

The Chiba Study of Mother and Children's Health (C-MACH) is a birth cohort study that has been conducted since 2014. The study is focused on the health effects of environmental factors (including chemicals) on fetuses and includes omics analysis to identify potential biomarkers and clarify these mechanisms.

In this article, we introduce the current findings of C-MACH, which aims to develop advanced preventive medical and strategic interventions during the fetal period that will help to lower the risk of chronic disease.

Keywords Birth cohort · Omics analysis · DOHaD · Environmental chemicals

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Abbreviations

BMI	Body mass index
DOHaD	Developmental origins of health and disease
HRM analysis	High-resolution melting analysis
ISO	International Organization for Standardization
NCD	Noncommunicable disease
PCB	Polychlorinated biphenyl
POPs	Persistent organic pollutants

10.1 Introduction

There are growing global concerns about infant, child, and adolescent health, with the increasing prevalence of noncommunicable diseases (NCDs) such as obesity and diabetes, neurodevelopmental disorders, allergies and respiratory diseases, and some cancers [1–3]. In previous studies, we have reported that environmental contaminants, such as polychlorinated biphenyls (PCBs), were detected in maternal blood, umbilical cords, and cord blood [4–6]. The early stages of human development are particularly sensitive to the effects of toxicants, which can interact with the processes of developmental plasticity. Recent epidemiological studies have shown that environmental factors during the fetal period and into early childhood might affect the risk of NCDs in adulthood [7–9]. If exposure can be prevented by scientific efforts, adverse effects on the health of future generations can also be prevented.

A cohort study is an epidemiological method that tracks groups of people with or without factors of interest, to clarify the relationship between those factors and outcomes. A cohort study that starts during pregnancy is called a birth cohort study. Well-designed, large-scale birth cohort studies will lead to improved understanding that can help to prevent possible adverse effects of environmental factors, such as chemical exposure, on infant, child, and adult health.

Recently, dramatic changes in social and living environments have led to an increase in NCDs. Epidemiological research focused in European countries [7–9] has revealed the effects of the nutritional environment from the fetal period to early childhood on NCDs in adulthood. The concept of developmental origins of health and disease (DOHaD) has been proposed [7, 10], which states that the risk of a variety of chronic diseases is affected by pre- and postnatal environmental factors such as maternal nutrition during the fetal period, lifestyle, stress, and exposure to environmental pollutants, as well as genetic sensitivity. Therefore, cohort studies are required to elucidate the effects of the fetal environment, including chemical exposure, on post-birth health issues [11].

Several mechanisms of the DOHaD concept have been proposed. One of these mechanisms is epigenetic changes to the genome caused by exposure to environmental factors during the fetal period and early childhood [10]; another is changes

in the gut microbiota of children, which might be affected by that of their mothers and the postnatal environment. Disruption of the gut microbiota balance (known as dysbiosis) affects the development not only of digestive system-related diseases but also of systemic conditions [12].

To evaluate these issues, omics analyses have been conducted in recent years. Epigenomic analysis, such as *epigenome*-wide association studies (EWAS), is applied to assess epigenetic changes; metagenomic analyses, such as 16S rRNA gene amplicon sequencing analysis, are used to assess gut microbiota. Additionally, metabolomic analysis, the exhaustive analysis of in vivo metabolites, has provided a more detailed understanding of changes occurring in vivo. Thus, the effects of environmental factors on health as well as the underlying mechanisms can be evaluated using these omics analyses.

A limited number of epidemiological studies have comprehensively used these novel analytical techniques and concepts. The analyses of epigenetic changes, gut microbiota, and the metabolome in epidemiological research might help to identify new biomarkers for predicting disease risk and determining the effects of environmental factors at an early stage.

In this article, we introduce the current findings of our cohort study, the Chiba Study of Mother and Children's Health (C-MACH), obtained using new research techniques such as omics, with the aim to develop advanced preventive medical and strategic interventions during the fetal period that will help to lower the risk of chronic disease.

10.2 About C-MACH

The C-MACH study was planned as a birth cohort study using omics analysis, including genome analysis, in addition to the aforementioned analyses to evaluate the effects of fetal and early childhood environmental factors on children's health [13]. C-MACH began in 2014 and is currently underway. The study consists of three hospital-based cohorts from Onodera Ladies Clinic and Yamaguchi Women's Hospital, both in Chiba Prefecture, Japan, and Aiwa Hospital in Saitama Prefecture.

The purpose of this study is to explore the effects of genetic and environmental factors, particularly the fetal environment and post-birth living environment, on children's health, and to identify biomarkers for these effects (Fig. 10.1). Primary outcomes of C-MACH are allergies, obesity, endocrine and metabolic disorders, and developmental disorders.

The following are our specific, approved research objectives:

1. Ascertain the links between nutritional intake by pregnant women and fetal development.
2. Establish biomarkers and indicators of epigenetic alteration to predict children's health problems, such as obesity, allergies, and impaired mental development.
3. Ascertain whether the interaction between fetal/neonatal environmental factors and genotypes is associated with mental development during childhood.

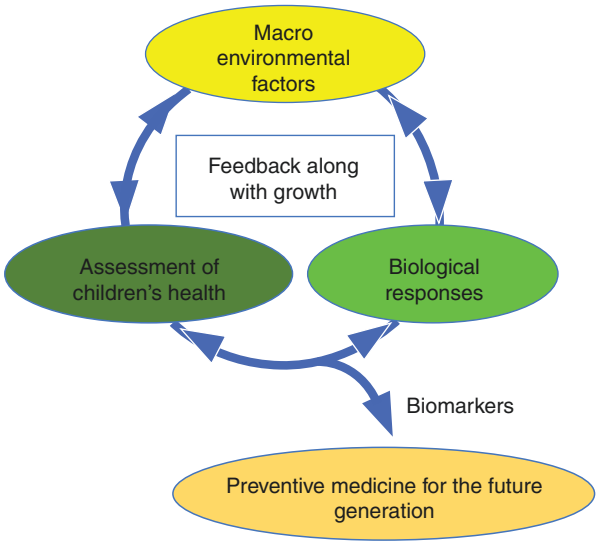

Fig. 10.1 Purpose and strategy of C-MACH

4. Ascertain whether the gut microbiota of mothers affects the development and health of their children, and clarify the factors that affect post-birth changes in gut microbiota.
5. Ascertain the effects of fetal exposure to environmental chemicals on epigenetic changes or the blood metabolome in children.

Pregnant women at less than 13 weeks' gestation were recruited. During pregnancy, participants underwent normal monitoring at the three hospitals. In the event of a stillbirth, participation in the cohort ceased for the mother. The recruitment population also consisted of all children born to women who consented to participate. The fathers of all recruited children were also candidates for participation. Participating women are withdrawn from the study if they are transferred to a different hospital for any reason.

Recruitment began in February 2014 and ended in June 2015. All participants provided written consent including completing questionnaire surveys and the collection, storage, and analyses of biological and home environmental samples.

All participants will be followed until the child reaches the age of 5 years. Follow-up will mainly be completed via questionnaire. Follow-up after the age of 5 years will be considered later.

During the first and last trimesters, questionnaires were administered, and blood, urine, and feces samples were collected. The questionnaire items include socioeconomic status, lifestyle habits, a brief diet history questionnaire, and psychological assessment.

We collected the usual medical findings at birth as well as umbilical cords, umbilical cord blood, placenta, and fecal samples. Data for the children are collected from health checkup records and questionnaires about child development and disease history at the ages of 1 month, 4 months, 10 months, 1.5 years, 3.5 years, and 5 years.

All biological samples are stored at −80 °C in the Chiba University Center for Preventive Medical Sciences BioBank. They will be preserved as biological specimens until the analysis is completed.

Until now, 434 women have provided their written consent to participate in C-MACH; 68 women withdrew after providing informed consent; as a result, 366 women are currently participating. We collected and analyzed questionnaires from 376 women in the early gestational period. The mean age of 376 participants was 32.5 (±4.4) years, and the mean age of women expecting their first child was 31.8 (±4.2) years. This was older than the mean age of the Japanese population (29.7 years) [14]. A total 98.4% of women were married, and 72.3% had an appropriate prepregnancy BMI (18.5–24.9 kg/m^2). Smokers during early pregnancy accounted for 5.0% of participants, which is lower than the percentage in other Japanese cohort studies [15–17].

10.3 Multilevel Analysis: Omics and Exposure

Omics analysis is a comprehensive method for analyzing various biological phenomena and includes several methodological approaches. Genomics targets genetic information, transcriptomics targets mRNA, and proteomics targets protein. Additionally, epigenetic alterations, metabolic changes, and microbiota are becoming target omics research fields; the analyses in these interesting fields are called epigenomics, metabolomics, and metagenomics, respectively. These comprehensive methods enable greater understanding of the mechanisms or risks of various diseases, to establish more precise medical approaches in the future.

Recently, these omics analyses are being performed in birth cohort studies, such as the Human Early-Life Exposome (HELIX) study and Tohoku Medical Megabank Project, among others [18, 19]. The DOHaD hypothesis proposes that interaction between genetic and environmental factors contributes to the onset of NCDs. Thus, it is useful to perform omics analyses in a birth cohort study, to detect the changes that appear before the onset of NCDs. Particularly, omics analyses targeting mothers, fetuses, and infants are important because of the fragility and plasticity of the fetus and infant.

In the C-MACH study, we plan to perform several omics analyses (Fig. 10.2): genomics, epigenomics, metabolomics, and metagenomics. Epigenetic changes, such as DNA methylation, are known to persist for a long period, thereby affecting human health and disease in adulthood.

10.3.1 Epigenetic Analysis

DNA methylation is one of the mechanisms hypothesized in the DOHaD model [20]. Comprehensive epigenetic analyses to investigate DNA methylation in the umbilical cord have been reported [21], with the use of a microarray (Illumina iScan system and Infinium HumanMethylation450 BeadChip, Illumina, San Diego, CA, USA) and methylation-sensitive high-resolution melting (HRM) analysis [22, 23].

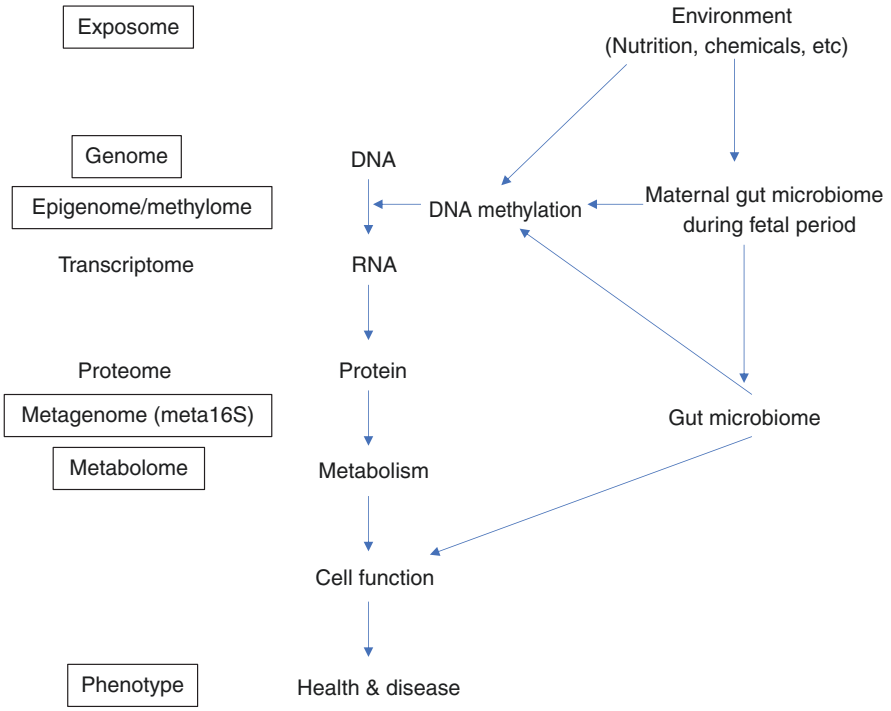

Fig. 10.2 The multilevel analysis of C-MACH. The analyses planned in C-MACH are indicated by solid lines

In C-MACH, we are examining variations in the DNA methylation of umbilical cords using a DNA methylation array. We will extract CpG sites that are correlated with outcomes, and detected candidate areas will be examined using HRM or pyrosequencing for all umbilical cord samples.

We performed pilot analysis to explore the relationship between umbilical cord DNA methylation and maternal factors. We found that DNA methylation of the umbilical cord at several CpG sites assessed by HRM or methylation array is associated with maternal factors. In one of these sites, methylation levels of the *H19* gene were associated with the mother's age [24]. The methylation levels of this gene may be regulated or affected by maternal aging, and it may cause some health effects in their children as this gene is important for fetal growth [25].

10.3.2 Metagenomic Analysis

The gut microbiome has come to be considered a novel environmental factor that affects our health [26–28]. Perturbations in the gut microbiome have been implicated in the cause of metabolic syndrome [29], and the role of the gut microbiome in pregnancy has become the subject of considerable interest [30].

Gut microbiota is analyzed using 16S rRNA gene sequences [31] or whole metagenomic sequences. In C-MACH, the DNA of gut microbiota is extracted from stool samples collected from mothers and their children, and we are analyzing the distribution and bias of gut microbiota using 16S rRNA sequence data.

We conducted a pilot study using maternal gut microbiota data. We did not detect any significant correlation between the proportion of the phylum *Firmicutes* and maternal anthropometric or nutritional parameters, such as maternal prepregnancy BMI, body weight gain during pregnancy, or caloric intake; however, these results might be owing to the small sample size. We then performed analysis combining maternal gut microbiota and epigenetic data. Surprisingly, a significant correlation between *Firmicutes* phylum of maternal gut microbiota and DNA methylation of CpG sites in diabetes-associated genes was found [31]. We are currently conducting this analysis using a larger sample size.

10.3.3 Metabolomic Analysis

Metabolome analyses have been used in toxicological and epidemiological studies to provide information about the biochemical status of a biological system [32]. Metabolome analysis can provide valuable insights relating to the biological responses to environmental changes [33, 34]. The composition of metabolites, such as amino acids, lipid metabolites, and vitamins in blood and urine, is analyzed using high-performance liquid chromatography/tandem mass spectrometry [35]. We are searching for biomarkers that reflect environmental exposure and studying the mechanisms for changes in metabolomes.

We reported our first results of metabolomic analysis in association with PCB concentrations. Citraconic acid in maternal serum and ethanolamine, *p*-hydroxybenzoate, and purine in cord serum were significant in the prediction model for classification of low versus high PCB concentration groups. There are several candidate biomarkers and metabolites included in composited models relating to glutathione and amino acid metabolism in maternal serum and compounds related to amino acid metabolism and ubiquinone and other terpenoid-quinone biosyntheses in cord serum [6].

10.3.4 Genomic Analysis

In the C-MACH study, genomic analysis will be conducted using maternal blood samples and saliva samples from participating children and partners. DNA will be extracted, and genome-wide association analysis using single-nucleotide polymorphisms will be performed to examine the genomic effects on outcomes, such as allergies.

10.3.5 Environmental Chemicals

We are assessing PCB levels in maternal and umbilical cord blood as an index of the exposure to persistent organic pollutants (POPs). We previously reported that PCB concentrations in blood and umbilical cord samples are correlated with the concentrations of various POPs [4, 5, 36–39]. Additionally, indoor dust samples have been collected and will be measured for house dust, mites, mold, and endotoxins to assess the effects of the indoor environment.

PCB levels are measured using gas chromatography-mass spectrometry [40, 41]. We have obtained International Organization for Standardization (ISO) laboratory certification for blood PCB measurement. In addition to total concentration, the composition of major individual congeners is assessed and the source of exposure inferred.

We reported that the association between diet habit and serum PCB concentration was assessed in our ISO-accredited laboratory. Interestingly, cooking methods were associated with serum PCB concentration in addition to foodstuffs [42]. Recently, we proposed new life course intervention including a virtuous cycle for reducing exposure to POPs for a healthier future [43].

10.4 Future Prospects and Conclusion

In this paper, we reviewed the profile and current findings of our birth cohort study with omics techniques, C-MACH. With the valuable results obtained from omics analysis of study participants' exposome, epigenome, microbiome, and metabolome, it may be possible to identify relationships between previously unknown social or environmental factors, such as certain pollutants and health risks. As a result of our birth cohort study, we hope that adverse health effects can be reduced to contribute to healthier future generations. Our mission and that of the current generation is to decrease adverse environmental effects so as to create a better living environment for all.

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