学 位 論 文 の 要 旨

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論 文 内 容 の 要 旨

INTRODUCTION

The general idea of the "Developmental Origins of Health and Disease" hypothesis, in which various environmental factors influence postnatal development and the risk of disease onset during adulthood, is intriguing. The concept is that future health and susceptibility to certain illnesses are strongly influenced by the prenatal and early postnatal environments. In addition, preemptive medicine, which provides early intervention for disease risk based on individual genetic backgrounds, has attracted increasing attention. In this context, it is important to focus on fetal factors that can influence neonatal telomere length (TL), which may be relevant for the development of cardiovascular diseases, diabetes, and malignancies in adults. TL is uniformly shortened during aging within the same organism, but differs between individuals. More recently, an association between maternal perinatal factors, fetal umbilical blood, and placental TL was reported in several unrelated studies. It is believed that individual differences in TL may be determined by the intrauterine environment, which is in turn affected by stress, diabetes, obesity, smoking, and small-for-gestational-age status. Thus, maternal health conditions and lifestyle habits may exert a significant influence on fetal programming, which could affect health in adulthood. Although some pregnant women have many of these risk factors, the factors that have a strong effect on TL have yet to be investigated. Therefore, in this study, we sought to define the major determinants of fetal TL.

MATERIALS AND METHODS

This study was conducted in a retrospective manner. The study protocol was approved by the Ethics Committee of Shimane University Hospital (approval no. 2020419-1). We focused on singleton pregnancies delivered at Shimane University Hospital between May 2016 and May 2018, and written informed consent for the analysis of neonatal TL and maternal perinatal factors was obtained from all participants. Eligible women were between 17 and 46 years of age. Among the 848 mother-newborn pairs examined in this period, 578 (68.1%) were ultimately included in the analyses. TL in cord blood samples were determined. Informed consent for the new analyses was obtained in the form of an opt-out on the registration website.

Eleven factors were analyzed for their association with neonatal TL: maternal age, ART, obesity, GDM, maternal stress, smoking, alcohol consumption, preterm delivery, small-for-gestational-age, neonatal sex, and placenta weight. We selected perinatal factors for telomere shortening with reference to previous literature. finding is controversial. Umbilical cord blood was drawn within 10 min after delivery and Peripheral blood mononuclear cells were isolated and stored at −20 °C. Genomic DNA was isolated and stored at −20 °C until analysis. DNA quantity and purity were assessed

Southern Blotting: The mean length of the telomere restriction fragment (TRF) was measured by partially modifying the method described by Slagboom et al.

Quantitative PCR: The method described by Cawthon et al. Before starting the experiment, we confirmed the correlation between the T/S ratio and telomeric restriction fragment length in 12 randomly selected neonatal samples. The T/S ratio was determined from 35 ng DNA with Fast Start Universal SYBR Green Master (Rox). The primer sequences were: tel1, 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′; tel2, 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′; 36B4u, 5′-CAGCAAGTGGGAAGGTGTAATCC-3′; and 36B4d, 5′-CCCATTCTATCATCAACGGGTACAA-3′. Triplicates of telomere runs showed a coefficient of variation (CV) of 0.62%, those of single-copy gene runs showed a CV of 0.65%, and those of T/S ratios showed a CV of 6.0% for cord blood.

Statistical Analysis : As the distribution of TL is biased, we performed base-10 logarithmic transformation on TL. For all factors, the data were analyzed using descriptive statistics. Log_{10} TL univariate analysis was performed using regression analysis for all factors. Multivariate regression analysis was performed for the relationship between TL and the 11 clinically important factors. All statistical analyses were conducted using Stata 15 (SPSS, Inc., Cary, NC, USA), with the threshold for statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

A total of 578 mother-newborn pairs were selected for TL analysis using quantitative PCR. The median neonatal TL to single-copy gene (T/S) ratio in cord blood was 1.0 (interquartile range, 0.7–1.5). The mean age of the pregnant women was 31.6 ± 5 (range, 17–46) years. Of the 578 pregnant women, 175 (30%) were older than 35 years. There were 58 women (10%) who became pregnant via assisted reproductive technology (ART), and 28 of these women used conventional in vitro fertilization, whereas 30 became pregnant by intracytoplasmic sperm injection. Mean body mass index (BMI) at 12 weeks was 21.3 kg/m^2. In terms of body weight, 97 women (17%) were underweight, 408 (71%) had standard weight, and 73 (13%) were obese. Forty-two women (7.3%) had gestational diabetes mellitus (GDM), 181 (31%) displayed a stress score ≥ 300 (i.e., high stress), 35 (6.1%) smoked throughout pregnancy, and 38 (6.7%) drank alcohol during pregnancy. Among the 578 newborns, 298 were male (52%); the mean gestational age was 38.6 (range, 29–42) weeks, and mean ($\pm$standard deviation) birth weight was 2962 ± 373.7 g. Thirty-eight newborns (6.7%) were preterm, 41 (7%) were small-for-gestational-age, and 187 (32.4%) had a small placenta.ART was among the 11 factors associated with shorter TL in cord blood. Multiple regression analysis showed that the TL of DNA in neonatal cord blood was shorter in the ART group ($p = 0.013$).

ART was identified as a factor associated with shorter neonatal TL using multivariate analysis. Among the various perinatal factors, ART may be strongly associated with neonatal TL. This is the first report to demonstrate that pregnancies with ART are associated with neonatal telomere shortening. Meister et al. reported that the incidence of hypertension is significantly increased during adolescence in individuals who were conceived by ART. Thus, based on previous reports and our present findings, the prognosis of children conceived by ART should be investigated carefully. Telomere shortening may result from oxidative stress, which is generated during ART procedures. We could not confirm the detailed conditions for ART in our survey, and it may be necessary to explore this in the future. Our findings suggest the need for the long-term investigation of children conceived by ART and for the examination of the correlation between oxidative stress and TL when using ART. The extent of oxidative stress generated when using ART, as well as its impact on TL in fertilized embryos and fetuses, should be investigated. Other ART factors that could be related to telomere shortening may need to be investigated, such as fertilized egg quality, patient's medical health status, and the effects of cytokines in the pelvis on infertility. In

CONCLUSION

Pregnancy by ART is associated with shorter TL in Japanese neonates. Given that pregnancy by ART is expected to continue increasing worldwide, the results of this study might be relevant to a new generation of human beings conceived by ART who could develop lifestyle-related diseases later in life. Therefore, further research in this field is urgently needed.