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Effects of adrenal androgens during the prenatal period on the second to fourth digit ratio in school-aged children

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ABSTRACT

Objectives: We investigated the relationship between the levels of adrenal steroid hormones in cord blood and the second to fourth digit ratio (2D/4D), which is regarded as an indirect method to investigate the putative effects of prenatal exposure to androgens, in school-aged children.
Materials and methods: Of the 514 mother-child pairs who participated in the prospective cohort study of birth in Sapporo between 2002 and 2005, the following adrenal steroid hormone levels in 294 stored cord blood samples (135 males and 159 females) were measured; cortisol, cortisone, androstenedione and dehydroepiandrosterone (DHEA). A total of 190 out of 350 children who were currently school-aged and contactable for this survey sent back photocopies of their palms for 2D/4D measurements.
Results: 2D/4D in all right hands, left hands, and mean values was significantly lower in males than in females ($p < 0.01$). DHEA levels were significantly higher in females. A multivariate regression model showed that 2D/4D negatively correlated with DHEA in males only ($p < 0.01$). No correlations were observed in the other adrenal steroid hormones tested in males or in any adrenal steroid hormones in females.
Conclusion: DHEA is mainly secreted in large amounts by the adrenal gland and is transformed into active sex-steroid hormones in peripheral tissues. The present study demonstrated that sex differences in digits were influenced by adrenal androgens during the prenatal period, possibly through intracrinological processes for androgen receptors located in fetal cartilaginous tissues.

1. Introduction

Exposure to sex hormones during the prenatal period is known to have an impact on sexual dimorphism. The extent of prenatal androgen exposure has been shown to affect differentiation to male-typical external and internal genitalia. Regarding digits, since androgen receptors are located in fetal cartilaginous tissues [1], the second to fourth digit ratio (2D/4D) is affected by the prenatal hormonal environment, such as exposure to higher levels of androgens and other gonad-specific hormones [2]. In humans, 2D/4D was reported to be smaller in males than in females [3], and is regarded as an indirect method to investigate the putative effects of prenatal exposure to androgens. We previously demonstrated that 2D/4D in school-aged children was affected by prenatal Leydig cell function in males [4]. Furthermore, this hypothesis as the underlying mechanism for differences in digits is supported by the following findings; lower 2D/4D in females with congenital adrenal hyperplasia (CAH) [5–7], higher 2D/4D in males with complete androgen insensitivity syndrome [8], and higher 2D/4D in men with Klinefelter's syndrome [9,10].

Abbreviations: CAH, congenital adrenal hyperplasia; DHEA, dehydroepiandrosterone; E, estradiol; INSL3, insulin-like factor 3; T, testosterone; 2D/4D, the second to fourth digit ratio.

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Androgens are mainly produced by Leydig cells in the testes of males, and small amounts are secreted by the adrenal glands. The ovaries of females also produce androgens, but to a lesser extent. By focusing on the adrenal glands, Labrie advocated the term 'intracrinology' in the field of endocrinology, which describes the local formation of active sex-steroid hormones [11]. In peripheral tissues, dehydroepiandrosterone (DHEA), as an inactive adrenal steroid precursor, is transferred into active androgens and estrogens by enzymes and then exert the important local effects of sex-steroid hormones [12,13]. Thus, in addition to the gonads, androgens derived from the adrenal glands also have the potential to affect sexual dimorphism during the prenatal period.

In order to elucidate the mechanisms underlying sexual differences in 2D/4D, hormone exposure needs to be measured during gestation, particularly in the earlier period of pregnancy. However, there is currently no established approach for measuring the hormonal environment earlier in pregnancy because of the ethical issues associated with normal pregnancy. Hormone levels in umbilical cord blood, which is obtained immediately after delivery, reflects a part of the hormonal environment of the fetus at late gestation [14,15]. Previous studies identified a relationship between fetal hormonal exposure and human development using cord blood [16–18].

Our previous study showed that no significant relation was identified between 2D/4D in school-aged children and testosterone, estradiol or progesterone in cord blood [4]. In the present study, we focused on androgens derived from the adrenal glands. Therefore, we investigated the relationship between 2D/4D in school-aged children and the levels of adrenal steroid hormones in cord blood.

2. Participants and methods

2.1. Participants

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Children's Health [19,20]. Study details regarding the population, data collection, sampling of biological specimens, and contents of the questionnaire have been described previously [19,20]. Briefly, native Japanese women living in Sapporo city or its surrounding areas were enrolled in the study at 23–35 weeks of gestation at Sapporo Toho Hospital between July 2002 and October 2005. Of the 1796 women approached, 25% were excluded because they decided to enroll in the Japanese cord blood bank or deliver the baby at another hospital; therefore, 514 pregnant women were enrolled in this cohort study (participation rate of 28.6%).

This study was approved by the Institutional Ethical Board for Epidemiological Studies at Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences. All participants provided written informed consent. Informed consent on behalf of the children enrolled was provided by their parents.

2.2. Measurement of 2D/4D

Ten out of 514 participants were excluded from the study due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before delivery. Since 7 sets of twins were born, a total of 511 children (246 males and 265 females) were finally included in the Sapporo Cohort study. Of these, 350 children (68.1%), who are currently school-aged and contactable for this survey, were requested via mail to send black-and-white photocopies of the palms of both the left and right hands. Measurements of digits were made from photocopies of the ventral surface of the right and left hands. Participants were instructed to straighten their fingers and lightly place their hands palm down on the photocopy machine. Measurements were made to the nearest 0.5 mm from the mid-point of the finger crease proximal to the palm to the tip of the finger using steel Vernier calipers. 2D/4D was calculated by dividing the length of the second digit by that of the fourth [3]. All measurements were taken twice by two observers blinded to participant information in order to confirm the measurements obtained as described previously [4].

2.3. Adrenal steroid hormone measurements in cord blood samples

At the time of delivery, a 10–30-mL blood sample was collected from the umbilical cord and stored at −80 °C for later analysis.

The following hormone levels in 294 stored cord blood samples (135 males and 159 females) were measured. Cortisol, cortisone, androstenedione, and DHEA levels were measured using LC-MS/MS [21,22]. All hormone measurements were performed by Aska Pharma Medical Co., Ltd. (Kanagawa, Japan).

2.4. Statistical analyses

Data on the characteristics of participants, 2D/4D, and sex hormone levels were presented as a group mean ± standard deviation and were analyzed between groups using a one-way ANOVA. Sex hormones were converted to a log10 scale as these data did not fall into a normal distribution. The relationship between 2D/4D and sex hormone levels in cord blood samples was calculated using a multiple linear regression analysis. The inclusion of covariates was based on biological considerations and adjustments were made for maternal age (continuous), birth weight (continuous), maternal smoking during pregnancy (yes or no), and maternal alcohol consumption during pregnancy (yes or no). All statistical analyses were performed using JMP pro 10 (SAS institute Inc., NC, USA), except for the intra-class correlation coefficient for right and left 2D/4D measurements, which was calculated using SPSS statistics version 19 (IBM, IL, USA). Significance levels were set to 0.05 for all comparisons.

3. Results

3.1. 2D/4D

A total of 190 children, including 88 males and 102 females, sent back photocopies of their palms. In all right hands, left hands, and mean values, 2D/4D was significantly higher in females than in males: 94.9 ± 0.3 vs. 93.2 ± 0.4 in right hands ($p = 0.0006$), 94.9 ± 0.3 vs. 93.5 ± 0.4 in left hands ($p = 0.0082$) and 94.9 ± 0.3 vs. 93.3 ± 0.4 in mean values ($p = 0.0006$), as described previously [4]. 2D/4D fell into a normal distribution in all right hands, left hands, and mean values. The mean 2D/4D value in both hands was used to determine its relationship with sex hormones as a representative value of each participant.

3.2. Adrenal steroid hormones in cord blood samples

The detection percentages of cortisol in males and females were 98.5% and 96.8%, while those of cortisone in males and females were 97.0% and 93.0%, respectively. The other adrenal steroid hormones were detected in all samples (Table 1). In samples with non-detected cortisol or cortisone level, a half of detection limit (DL) was used as a value of cortisol or cortisone level in data analysis. The intra-assay and inter-assay coefficients of variations in terms of adrenal steroid hormone measurements were as follows; cortisol: 3.9%–10.9%, Cortisone: 1.3%–9.9%, androstenedione: