**Supplementary table S1. Characteristics of TIDES study participants including UCSF center (n=424).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Missing (n)** | **Total sample (n=424)** | **Males (n=200)** | **Females (n=224)** |
|  |  | Mean ± SD | Mean ± SD | Mean ± SD |
| **Continuous variables (maternal)** |  |  |  |  |
| Age (years) | 1 | 31.48±5.43 | 31.16±5.29 | 31.77±5.55 |
| Maternal hormones  Estriol (E1)  Estradiol (E2)  Estrone (E3)  Free testosterone (fT)  Total testosterone (TT) | 86  86  88  89  84 | 968.80±907.43  1656.34±1266.32  161.85±259.05  0.33±0.21  70.48±42.83 | 917.55±875.78  1646.01±1355.98  167.32±264.20  0.33±0.23  70.64±45.05 | 1014.33±934.74  1665.53±1184.77  156.94±254.99  0.33±0.20  156.94±254.99 |
| Gestational age at blood collection (days) | 81 | 76.95±17.80 | 75.92±18.68 | 77.85±17.01 |
| Parental attitudes about gender-atypical play  Boys (PAB)  Girls (PAG) | 2  2 | 5.68±1.79  4.91±1.61 | 5.70±1.87  4.98±1.67 | 5.65±1.71  4.85±1.56 |
| **Continuous variables (child)** |  |  |  |  |
| Age at infant exam (days) | 0 | 5.73±14.26 | 6.40±13.90 | 5.13±14.57 |
| Body size at infant exam (weight for length z-score) | 4 | -0.45±1.32 | -0.55±1.25 | -0.36±1.37 |
| Infant AGD-AP (boys)/AGD-AC (girls) | 2 | - | 49.41±5.54 | 36.57±3.67 |
| Infant AGD-AS (boys)/AGD-AF (girls) | 0 | - | 24.40±4.27 | 16.11±3.06 |
| Age at digit measurement (years) | 0 | 4.51±0.32 | 4.50±0.33 | 4.52±0.31 |
| Digit length and ratio  2nd digit (mm)  4th digit (mm)  2D:4D | 0  0  0 | 44.95±3.10  46.41±3.46  0.97±0.07 | 45.31±3.03  46.87±3.28  0.97±0.06 | 44.62±3.14  45.99±3.58  0.97±0.07 |
| Gender-typed play behavior  Composite  Masculine sub-scale  Feminine sub-scale | 17  8  11 | 49.51±17.13  37.06±8.22  36.10±10.38 | 64.32±8.92  42.27±6.26  27.64±5.75 | 35.89±10.12  32.43±6.87  43.97±6.97 |
| Siblings  Number of brothers  Number of sisters | 0  0 | 0.58±0.70  0.54±0.66 | 0.58±0.70  0.50±0.60 | 0.58±0.70  0.58±0.70 |
| **Categorical variables** |  | N (%) | N (%) | N (%) |
| Maternal education  Less than college graduate  College graduate or higher | 2 | 96 (22.64)  326 (76.89) | 45 (22.50)  154 (77.00) | 51(22.77)  172(76.79) |
| Child race  White  Other | 0 | 269 (63.44)  155 (36.56) | 130 (65.0)  70 (35.0) | 139(62.05)  85(37.95) |
| Study Center  UCSF  UMN  URMC  UW | 0 | 103 (24.29)  122 (28.77)  115 (27.12)  84 (19.81) | 46 (23.0)  62 (31.0)  56 (28.0)  36 (18.0) | 57 (25.45)  60 (26.79)  59 (26.34)  48 (21.43) |

**Supplementary table S2.** **Digit ratio1 in relation to anogenital distance (Models 1-4), sex (Model 5), and covariates with all study centers (including UCSF).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Female** | | **Male** | | **Both sexes** |
|  | **Model 1**  **(n=220)** | **Model 2**  **(n=221)** | **Model 3**  **(n=198)** | **Model 4**  **(n=154)** | **Model 5**  **(n=424)** |
|  | β (95% CI) | β (95% CI) | β (95% CI) | β (95% CI | β (95% CI) |
| **AGD-AC (female)5** | 0.40 (-0.67, 1.46) |  |  |  |  |
| **AGD-AF (female)5** |  | 0.39 (-1.02, 1.80 |
| **AGD-AS (male)5** |  | 0.24 (-0.69, 1.16) |
| **AGD-AF (male)5** | 0.20 (-0.57, 0.98) |  |
| **Sex2** |  | 1.30 (-3.83, 6.57) |
| **Child age at digit measurement** | 3.52 (-8.83, 15.87) | 4.35 (-8.33, 17.03) | -7.85 (-20.22, 4.52) | -7.46 (-19.81, 4.90) | -1.67 (-10.14, 6.81) |
| **Race3** | -3.71 (-11.85, 4.44) | -3.4 (-11.53, 4.69) | -6.46 (-14.79, 1.87) | -6.35 (-13.67, 1.96) | -4.16 (-9.82, 1.50) |
| **Study center4**  **URMC**  **UW**  **UCSF** | -5.16 (-15.93, 5.61)  -5.51 (-16.51, 5.49)  22.29 (11.88, 32.70) | -5.15 (-15.95, 5.64)  -6.77 (-18.23, 4.69)  23.00 (12.36, 33.63) | -0.65 (-10.99, 9.68)  4.94 (-7.34, 17.21)  17.67 (7.04, 28.30) | -1.09 (-11.32, 9.13)  3.07 (-8.89, 15.02)  17.45 (6.81, 28.08) | -3.81 (-11.12, 3.49)  -2.38 (-10.25, 5.50)  19.46 (12.21, 26.7) |

1 Dependent variable (digit ratio) is log10 transformed and multiplied by 1000.

2 Reference=male

3 Reference=white

4 Reference=UMN

5 Residual AGD after adjusting for age at exam and weight for length z-score.

**Supplementary table S3. Adjusted linear regression models examining maternal hormones1 in relation to child digit ratio in all TIDES participants (including UCSF site) 2,3.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **n** | **Male4**  **β (95% CI)** | **Female4**  **β (95% CI)** |
| **Estrone (E1)** | 338 | 8.56 (-4.35, 21.48) | 6.44 (-5.44, 18.32) |
| **Estradiol E2)** | 338 | 2.06 (-13.59, 17.71) | -0.40 (-15.19, 14.39) |
| **Estriol (E3)** | 336 | -8.10 (-21.38, 5.18) | 3.29 (-8.47, 15.04) |
| **Free testosterone (fT)** | 335 | 6.11 (-10.36, 22.58) | -29.63 (-46.48, -12.78) |
| **Total testosterone (TT)** | 339 | 7.30 (-10.01, 24.67) | -5.37 (-23.65, 12.91) |

1 All hormones are adjusted for gestational age at blood collection and log10 transformed.

2 Dependent variable (digit ratio) is log10 transformed and multiplied by 1000.

3 Adjusted for child sex, sex\*hormone, child age at digit measurement, race, study center

4 Models were reparameterized to provide coefficients for the sex-specific slopes

**Supplementary table S4. Adjusted linear regression models examining child digit ratio in relation to gender-typed play behavior (PSAI scores)1 in all TIDES participants (including UCSF site).**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **n** | **Male2**  **β (95% CI)** | **Female2**  **β (95% CI)** |
| **PSAI composite score** | 403 | 2.38 (-19.55, 24.31) | -3.09 (-23.27, 17.09) |
| **Masculine sub-score** | 412 | -2.19 (-17.21, 12.83) | -3.05 (-16.70, 10.57) |
| **Feminine sub-score** | 409 | -4.08 (-18.5, 10.36) | 1.86 (-11.11, 14.83) |

1 Adjusted for child sex, maternal education, child’s race, parental attitudes about gender-atypical play for the gender of the child, number of brothers, number of sisters,

2 Models were reparameterized to provide coefficients for the sex-specific slopes

**Supplementary figure S1.** Digit ratio by study center in TIDES.

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