Resolving the role of prenatal sex steroids in the development of digit ratio

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Prenatal sex steroids [i.e., prenatal testosterone (PT), prenatal estrogen (PE)] are often implicated in the etiology of behaviors and diseases. They show sex differences (higher concentrations of PT to PE in males), with PT peaking at the end of the first trimester, and cause permanent "organizational" changes in the brain and other organ systems. It has been suggested that relative levels of PT and PE may have differential effects on fertility, speed, strength, aggression, autism, many cancers, and heart disease. However, PT and PE are difficult to assess, and this has prevented the establishment of convincing links. The relative lengths of the second and fourth digits [digit ratio (2D:4D)] is thought to correlate negatively with PT and positively with PE, thus affording us the possibility of establishing such links. Although associations between 2D:4D and many sexually dimorphic traits have been investigated, the developmental link between 2D:4D and PT and PE has remained controversial until now. In PNAS, Zheng and Cohn (1) present an elegant and powerful experimental examination of the influence of PT and PE on the development of 2D:4D in the mouse. To appreciate the relevance of their findings, it is necessary to consider them in the context of the recent history of 2D:4D research.

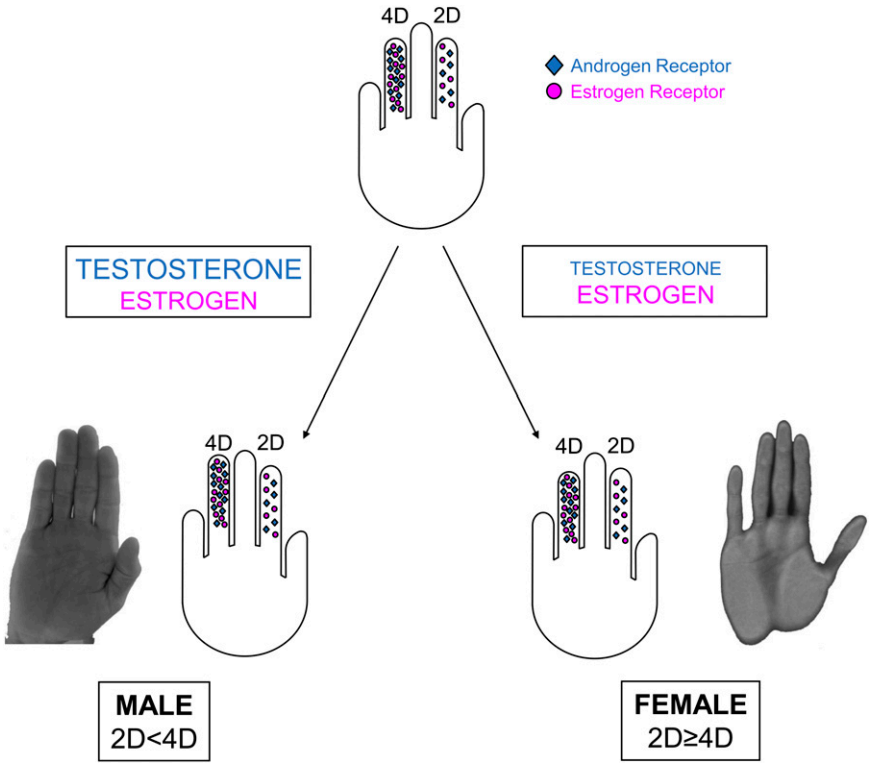

Fig. 1. Causal links between PT, PE, and 2D:4D. As described by Zheng and Cohn (1), receptors for androgen and estrogen are present on digits 2 and 4 of the mouse but are most plentiful on the fourth digit. Chondrocyte proliferation is stimulated by PT but arrested by PE. Compared with females, males have higher levels of PT relative to PE and longer fourth digits relative to second digits. Thus, the sex difference in 2D:4D is dependent on prenatal changes in the fourth digit caused by sex-dependent differences in the PT-to-PE ratio. Examples of male and female human hands are shown for comparison.

Recent History of 2D:4D Research

The sexual dimorphism in 2D:4D (males generally have longer fourth digits relative to second digits than females) was first noted in the 19th century (2). However, it was not until 1998 that 2D:4D was hypothesized to be (*i*) linked to genes that influence the formation of limbs and the urogenital system and (*ii*) negatively correlated to PT and positively correlated to PE (3). These suggestions stimulated 2D:4D research, such that the annual numbers of 2D:4D papers increased from 1 to 51 from 1998 to 2007 and the numbers have averaged about 60 papers per year from 2008 to 2010.

Correlation Studies

Progress has been rapid in describing the phenotypic patterns of 2D:4D and its correlations to sex-dependent traits. Early studies that have stood the test of replication have reported that 2D:4D varies by sex and ethnicity but that male 2D:4D tends to be smaller than female 2D:4D in all ethnic groups and the effect is strongest in the right hand (4); in general, mammalian 2D:4D shows similar sex differences (5); the sexual dimorphism in 2D:4D arises early in fetal development (6); 2D:4D is a strong negative correlate of sports ability (7), and this relationship extends to other activities with competitive characteristics [e.g., short-term financial trading (8)]; low 2D:4D is linked to left-handedness (9) and developmental disorders, such as autism (10); and 2D:4D may be a biomarker for some sex-dependent diseases, such as osteoarthritis (11). However, these correlation studies have raced ahead of evidence that 2D:4D is dependent on PT and PE. In this regard, most studies have focused on correlations with PT (12), whereas only a few have considered both PT and PE (13). Of importance to the former are correlations of 2D:4D with traits that alter the normal levels of PT. Congenital adrenal dysplasia is one such trait, which is associated with high prenatal androgen and low 2D:4D (14, 15). In addition, high sensitivity to testosterone has been reported to be correlated with low 2D:4D (16, 17). However, other studies have reported null findings with regard to correlation studies between 2D:4D and sensitivity to androgen (18) and have challenged the correlation between 2D:4D and PT (19). Clearly, we need experimental studies that do not suffer from the limitations associated with correlation data. There has been one such study, which showed that enhancement of PT

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reduces 2D:4D in rats (20). However, if both PT and PE are important in determining 2D:4D, variation associated with the former may be obscured by the latter. Therefore, we must consider the influence of both PT and PE on variation in 2D:4D.

Developmental Studies

The work of Zheng and Cohn (1) illustrates the power of experimental studies to resolve the problems associated with correlation data. In essence, they show that 2D:4D is determined by the balance of PT to PE signaling in a narrow window of fetal digit development. Using an early molecular marker (*Sox9*) for cartilage determination in the digits, they found that fetal sex differences in mouse 2D:4D are established between embryo day (E) 12.5 and E17. Interestingly, paralleling human observations, this effect appeared to be more powerful for the right paw. They then demonstrated that the developing digit primordia were rich in androgen (AR) and estrogen (ER) receptors, and that this was particularly so in the fourth digit. Inactivation of AR and ER and the use of receptor antagonists (flutamide and fulvestrant), dihydrotestosterone, and estradiol showed that 2D:4D was determined by a balance of PT and PE acting on the fourth digit; that is, AR increased chondrocyte proliferation, whereas ER reduced chondrocyte proliferation in the fourth digit. Thus, the sex difference in 2D:4D arises from higher values of PT relative to PE found in males compared with females, and this effect is driven by changes in length of the fourth digit. Importantly, receptor antagonists and hormones applied after birth did not influence 2D:4D, but they did affect anogenital distance. Therefore, 2D:4D is fixed in the embryo but anogenital distance is not.

Thus, it turns out that 2D:4D is determined not by PT alone but by the balance of PT to PE signaling in a narrow time window of fetal digit development. The general failure to take into account the influence of PE, together with the lack of power of correlation studies, has obfuscated real relationships between prenatal sex steroids and 2D:4D. As for the future, Zheng and Cohn (1) have provided us with a list of 19 genes that are sensitive to PT or PE and are involved in the formation of the phalanges in the fourth digit. Armed with this list of skeletogenic genes linked to 2D:4D, we can now be more focused in our examination of the links between 2D:4D and the etiology of sex-dependent behaviors and diseases of the immune system, cardiovascular disorders, and a number of cancers.

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