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# Re-examining the Manning hypothesis: androgen receptor polymorphism and the 2D:4D digit ratio

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**Abstract**

The 'Manning hypothesis,' the idea that small differences in the ratio of the lengths of the human second to fourth digits—the 2D:4D ratio—reflect differences in the level of fetal androgen exposure, has been highly influential in the biological and biobehavioral sciences. The ratio is widely used to investigate the involvement of fetal androgens in the differentiation of sexually dimorphic traits. The validity of such studies is based on the premise that individual differences in the size of the 2D:4D ratio mirror differences across individuals in developmental levels of androgen exposure in a dose-dependent manner. Despite its widespread adoption by researchers, clinical evidence has yet to confirm that individual gradation in the ratio denotes differences in testosterone action. Key support for the view that 2D:4D does, in fact, reflect fetal testosterone in a graded fashion is the finding, based on a single small-sample study, that the magnitude of 2D:4D covaries with a polymorphic repeat (CAG) sequence in exon 1 of the gene coding the androgen receptor, *AR*. In a larger independent sample, we reexamine this genetic association and fail to substantiate a correlation between *AR* CAG length and 2D:4D. Combined with other recent reports, these data question one of the fundamental pieces of evidence on which the Manning hypothesis rests and raise new issues regarding the extent to which 2D:4D is a valid reflection of differences in fetal testosterone action in normally developing individuals.


*Keywords:* Organizational effect; Prenatal; Sex difference; Testosterone; CAG repeat

## 1. Introduction

The 2D:4D digit ratio—the ratio of the lengths of the second to fourth digits of the human hand—is a sexually dimorphic trait (Ecker, 1875). Although it varies considerably across individuals, men on average have a lower 2D:4D ratio than women. Interest in the ratio was triggered in 1998 when British researcher John Manning hypothesized that the sex difference in 2D:4D is determined by actions of testosterone in utero and that individual variation in the size of the ratio may be a proxy for individual differences in the level of fetal androgen exposure (Manning, Bundred, Newton, & Flanagan, 2003; Manning, Scutt, Wilson, & Lewis-Jones, 1998). Prenatal androgens are believed to masculinize the human central nervous system (Hines, 2011). Consequently, the prospect that 2D:4D represents a retrospective marker of the level of androgen exposure during the period when the brain is sexually differentiated has led to nearly 500 studies in the biosciences investigating links between the size of the ratio and variation in sex-related traits believed to be androgen-dependent ranging from autism, to fertility, to behavioral characteristics such as sexuality, risk-taking, or aggression (for review, see Manning, 2008).

The scientific integrity of these studies is based on the premise that individual variation in the ratio constitutes a valid marker of androgen action. The validity of 2D:4D, however, is contested. In favor of an androgen effect, the ratio is reduced (more male-typical) in patients with 21-hydroxylase deficiency who experience elevated androgens in utero (Brown, Hines, Fane, & Breedlove, 2002; Ökten, Kalyoncu, & Yariş, 2002) and is female-typical in 46,XY patients with complete androgen insensitivity (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009), in whom the androgen receptor (AR) fails to bind testosterone. These data

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confirm gross changes in the ratio in response to large deviations in androgen availability but do not confirm that small gradations in 2D:4D, visible among healthy persons of either sex, mirror fetal androgen exposure in a dose-dependent fashion. Variation in the ratio within each sex could potentially be an expression of multiple causative factors, not limited to or even largely reflective of androgen action (e.g., Zheng & Cohn, 2011). The view that individual variation in the ratio reflects androgen exposure in a graded, proportional manner is based largely on a single study that appeared in *Evolution and Human Behavior*. Manning et al. (2003) discovered that the size of the 2D:4D ratio covaries, across healthy males, with a repeat polymorphism in the gene that encodes the AR [number of polyglutamine (CAG) repeats in exon 1 of the *AR* gene], which is known to correlate inversely with the ability of the receptor to activate transcription of androgen-dependent target genes (Zitzmann, 2009). Manning reported that 2D:4D correlated positively with *AR* CAG length, supporting the view that individual differences in the ratio are a meaningful reflection of differences in the level of testosterone action.

Despite its theoretical importance, the Manning et al. (2003) result is based on only a single small sample of 50 men and has yet to be replicated. Recent work by Medland et al. (2010), using genomewide screening, and by Hurd, Vaillancourt, and Dinsdale (2011) has cast some doubt on the CAG association. In a study of aggressive behaviour, both 2D:4D and *AR* CAG length were measured by Hurd et al. (2011), but *AR* CAG length failed to show the anticipated correlation with 2D:4D (right hand: $r$=.006; left hand: $r$=−.12). Medland et al. (2010) failed to find any significant genetic association between 2D:4D and four different single nucleotide polymorphisms (SNPs) located within the *AR* gene, although CAG repeat expansion per se was not specifically measured.

Here, we report data in which *AR* CAG genotype was specifically quantified in a large sample of healthy young men in whom a dedicated measure of the 2D:4D ratio was taken. To evaluate the possibility that genetic variation in *AR* could lead to covariation in the production of testosterone via physiological feedback mechanisms (Crabbe et al., 2007)—which potentially could obscure any correlation—we also measured bioavailable testosterone in the same sample.

## 2. Methods

### 2.1. Participants

The current work was part of a larger investigation of associations between testosterone biomarkers, affect, and cognition. Participants were 152 physically healthy males recruited from the University of Western Ontario ($M$=18.72 years; S.D.=1.63; range=17–27 years). The sample was predominantly Caucasian; 10% of the sample was Asian. Participants were excluded if they reported an injury to their hands that could alter the growth of their second or fourth digits or if they had any known endocrine pathology.

### 2.2. Genotyping

Genotyping was performed by The Center for Applied Genomics at the Hospital for Sick Children in Toronto. Briefly, participants collected 2 ml of saliva into a sterile Oragene·DNA vial. To ensure the purity of the saliva, participants abstained from eating, drinking fluids other than water, smoking, chewing gum, or brushing their teeth for 30 min prior to sample collection. Once collected, the whole saliva was mixed with 2 ml of Oragene·DNA stabilizing solution and capped. Using a 50-ng DNA extract, the CAG repeat region of the AR gene was amplified via polymerase chain reaction with one primer labeled with 6-FAM dye for visualization (5′-CTTTCCAGAATCTGTTCCAG-3′) and a second unlabeled primer (5′-GAAGGTTGCTGTTCCT-CATC-3′). Amplified fragments were run through capillary electrophoresis and read using an ABI3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Quantification of the length of the CAG repeat region from each sample was accomplished using GeneMapper v.3.5. Repeat numbers (CAGn) were confirmed by sequencing a reference subset of samples with alleles of different lengths. Eighteen men (12%) could not be genotyped due to limited quality or quantity of DNA available.

### 2.3. Digit ratios

Digital images of the underside of both the right and left hands were taken with fingers placed in a splayed position and hands lightly pressed to distribute pressure evenly over the surface of the hand. Finger length was measured from the most basal crease where the finger joins the palm to the most distal point on the fingertip using a high-precision digital calliper (Digital Measurement Metrology, Inc., Model ABS) with a resolution of 0.005 mm. Digit ratios were computed for the right hand, left hand, and, following Manning et al. (2003), the difference between the right and left ratios. Finger lengths were independently scored by a second rater who was blind to the other's measurements. Interrater reliabilities, calculated using intraclass correlation, were as follows: right hand ratio IC$r$=.94, left hand ratio IC$r$=.95.

### 2.4. Testosterone

Testosterone was measured from saliva. Unlike serum or plasma, saliva affords a direct index of the 'bioavailable' fraction of the testosterone in circulation, i.e., the fraction of the total hormone that is metabolically active and available to tissue (Pardridge & Demers, 1991). In contrast, a substantial fraction of the testosterone detectable in serum is bound to sex hormone binding globulin and is physiologically inert. Specimens were collected between 1300 and 1900 h, a time of day when concentrations are stable (Gupta, Lindemulder, & Sathyan, 2000) and which is recommended for studies where individual differences in testosterone are a focus of