Personality and Individual Differences 52 (2012) 224–227

Contents lists available at SciVerse ScienceDirect

Personality and Individual Differences

journal homepage: www.elsevier.com/locate/paid

Short Communication

Is CAG sequence length in the androgen receptor gene correlated with finger-length ratio?

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ARTICLE INFO

Article history:
Received 11 March 2011
Received in revised form 12 September 2011
Accepted 13 September 2011
Available online 17 October 2011

Keywords:
Finger-length ratio
Androgen receptor
2D:4D

ABSTRACT

Manning, Bundred, Newton, and Flanagan reported a significant correlation of .29 in a sample of 50 British males between the length of a repeated sequence on the androgen receptor gene and 2D:4D finger-length ratio on the right hand. We report a 2nd failure to replicate this result. Ours was a sample of 182 Australian male twins studied for other purposes, for whom both measures were available. The result was a nonsignificant correlation of −.055. A similar result was obtained for female twins, and for comparisons within sibling pairs. Correlations are also reported for left hands and right–left differences––the last showed a weak tendency toward replication.

John Manning and his colleagues (Manning, Bundred, Newton, & Flanagan, 2003) reported a significant positive correlation of .29 in a sample of 50 British men between the second- to fourth-finger ratio (2D:4D) on the right hand and the length of a repeated CAG sequence in the androgen receptor (AR) gene. Longer CAG sequences have been related to less effective transcription of testosterone (Chamberlain, Driver, & Miesfield, 1994). Thus the Manning et al. finding suggests that differences in sensitivity to androgens, as well as differences in androgen levels themselves, may be playing a role in determining the 2D:4D ratio. This would not explain sex differences, for which CAG sequence lengths do not differ, but might be related to individual differences within a given sex. In a recent review, Breedlove (2010) makes Manning's correlation a centerpiece in his argument that digit ratios reflect prenatal androgens:

> "The strongest evidence that androgens affect digit ratios is the report that normal polymorphism in the *androgen receptor (AR)* gene correlates with digit ratios in men."
> (p. 4117)

The 2D:4D literature is replete with failures to replicate correlational relationships discovered in small samples (e.g. <100 cases). We are aware of one other effort to replicate this particular one (Hurd, Vaillancourt, & Dinsdale, 2011). It did not succeed: the authors found a correlation of only .006 between AR and right-hand 2D:4D in 180 male Canadian undergraduates. At the time we learned of the Hurd et al. article, we had been carrying out an attempt at replication in an Australian sample of twins and their siblings that had been assembled for other purposes by Martin and his colleagues (Wright & Martin, 2004). For a subgroup of this sample, both digit ratios and AR gene repeats were available. Thus we could ask: What is the correlation between right-hand digit ratios and AR repeats for the males in this group? The Australian males were younger than Manning's British males (mean age 17.9 as against 32.6 years)––as were the undergraduates in the Hurd et al. study (mean age 19.2 years)––but for a stable effect determined prenatally, age differences should not matter.

It should be emphasized that we are not here addressing the broader issue of whether prenatal androgens are related to individual and sex differences in finger-length ratios. We are here concerned with a more limited question: are differences in the sensitivity to androgens, as reflected in the length of the repeated CAG sequence on the AR gene, associated with (and hence possibly responsible for) individual differences in finger-length ratios?

In addition to right hands, Manning et al. (2003) reported for their sample the correlation of AR sequence length with left-hand 2D:4D (a trivial .005) and with the difference in 2D:4D between right and left hands (a significant .36). Hurd et al. obtained non-significant correlations for these of −.12 and .13, respectively. These correlations also are available in the Australian sample. Because the Australian sample also contains females, we can compare results for them. Males are exposed prenatally to higher levels of androgens than females, and this is hypothesized to lead to their lower average 2D:4D ratios, but if differing finger-length ratios for individuals within each sex result from differing prenatal

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doi:10.1016/j.paid.2011.09.009

androgen levels (and/or different levels of sensitivity to androgens), we would expect comparable patterns of correlation within the two sexes. The AR gene is X-linked, so females have two AR genes whose CAG sequences may be of different length, whereas males have only one. However, for females one copy of the gene is inactivated within each somatic cell, apparently at random. Thus, for the females, we use the mean AR sequence length of the individual's two alleles.

Finally, because the Australian sample consists of twins and siblings, we can examine the relationships within sibships: Does the difference in length of two siblings' AR sequences predict their difference in digit ratios? Such a within-sibship comparison equates for many variables left uncontrolled in an overall comparison, including ancestry, parental traits, maternal hormonal characteristics, and any other variables that siblings share.

1. Method

1.1. Participants

The participants consisted of twins and siblings close to them in age. The sample available for this study consisted of 400 individuals, 45 of whom were monozygotic (MZ) twins, 279 dizygotic (DZ) twins, and 76 siblings. They came to the Queensland Institute of Medical Research for testing at three ages: approximately 12, 14, and 16 years. Some came at all three ages, some at one or two. Overall, the study families were predominantly of Caucasian ancestry: 68% of the grandparents were British or Australians of British descent, and a further 19% came from other Caucasian populations.

For the within-pairs comparisons, MZ pairs as such were excluded, because their AR genes do not differ, but pairings of an MZ twin with a sib were included.

1.2. Measures

A blood sample was taken at one visit, and was the basis for assessing the number of repeats of the CAG triplet on the AR gene. Participants in the study were genotyped by the Australian Genome Research Facility (Ewen et al., 2000) as part of a linkage study for a range of cognitive and physiological traits (see Zhu et al., 2004 for a description of the protocol). A photocopied image of the two hands was taken at one or more of the visits. Participants who had gone through the testing before the photocopying was added were asked to make photocopies of their hands and send them in by mail. About half of the available photocopies fell in this category. In either case, the lengths of the 2nd and 4th fingers on each hand was measured in the laboratory from the photocopy, and a 2D:4D ratio calculated.

Four hundred individuals (399 for the left hand) had both digit ratio and AR measures, and constitute the basic sample for the present study. Some had their hands photocopied on more than one occasion (73 twice, 8 three times). For these, 2D:4D ratios were obtained for each occasion and averaged.

2. Results

First, did we confirm Manning et al.'s (2003) finding of a significant positive correlation in males between right hand 2D:4D and AR sequence length, or did we replicate Hurd et al.'s (2011) finding of a trivial one? The latter. For the 182 males in the Australian sample, the correlation between right-hand 2D:4D and AR sequence length was −.055. This is not significantly different from the near-zero Hurd et al. correlation, and is in the wrong direction for the hypothesis. These and other correlations from the three studies are summarized in Table 1.

The table suggests little evidence of replication of the Manning et al. (2003) results. For males, none of the Canadian or Australian correlations with left- or right hand 2D:4D was statistically significant, and those in the wrong direction for the hypothesis (negative *r*s) tended to be of comparable magnitude to those in the expected direction. A possible exception was the correlation with the right–left difference in 2D:4D. Although not statistically significant in the Canadian or Australian samples, it was positive in both. Controlling ethnicity (Canadian sample) or all between-family variables (Australian sample) left these conclusions essentially unchanged.

The females in the Australian sample yielded a lack of significant correlation with AR for right-hand, and right minus left-hand finger-length ratios, the ones that had been significant for males in Manning's original study. For left-hand ratios, where Manning found a near-zero correlation, and the Canadian and Australian male correlations had been nonsignificantly negative, there was a small but statistically significant positive correlation for females.

3. Discussion

Obviously, a single failure to replicate––or even two––does not permanently rule out a relationship, but it might suggest caution in leaning heavily upon it in constructing theory; note that the Canadian and Australian male samples of 180 and 182 were nearly four times the size of Manning's 50 males.

For Australian females, the same null result was found for the right hand as for males, but there was a just-significant positive correlation on the left hand. Because it was numerically small, was not postulated *a priori*, and disappeared in the within-pairs comparison, it is probably appropriate to regard it with some skepticism until replicated in other samples.

Was there anything amiss in the AR measurement or finger-length ratios in the present study that might explain these differences? The mean AR sequence lengths were 20.6 and 20.9 for males and females, respectively. Judging from the figures in Manning's paper, the two most common sequence lengths in his sample were 20 and 21 (Manning et al., 2003). In Manning's study, finger lengths were measured directly from the hands, in Hurd et al.'s from photographs, in ours from photocopies. Different methods for measuring finger lengths sometimes show differences in means (e.g. Almasry, El Domiaty, Algaidi, Elbastawisy, & Safwat, 2011; Manning, Fink, Neave, & Caswell, 2005), but correlational

Table 1
Correlations in three studies between the number of androgen receptor gene CAG repeats and 2D:4D finger-length ratios.

Sample	*N*	2D:4D finger-length ratio		
		Right-hand	Left-hand	Right–Left
Males–overall				
British	50	.29 (.04)	.005 (.98)	.36 (.01)
Canadian	178–80	.01 (.93)	−.12 (.11)	.13 (.07)
Australian	181–2	−.06 (.46)	−.13 (.09)	.10 (.20)
Males-within ethnicity				
Canadian	157	.05 (.52)	−.08 (.32)	.14 (.08)
Males-within sib pairs				
Australian	68–9	.06 (.62)	−.10 (.42)	.19 (.12)
Females, Australian				
Overall	218	.08 (.24)	.14 (.04)	−.06 (.36)
Within sib pairs	90	.14 (.18)	.06 (.58)	.11 (.32)

Note: British sample, Manning et al. (2003); Canadian sample, Hurd et al. (2011) ("Within ethnicities" based on two largest groups, Caucasians, *N* = 102 and Asians, *N* = 55); Australian samples, present study (*N* for within sib pairs = number of pairings). Probabilities (in parentheses) are based on 2-tail tests throughout. Those for the British sample were converted from the 1-tail probabilities reported in Manning et al. (2003); those for the Canadian males within ethnicities are approximated from the *N*s and *r*s reported by Hurd et al. (2011).

agreement tends to be good (Voracek & Dressler, 2006). Our sex difference was typical––mean 2D:4D ratios significantly higher for females. Also present was a greater sex difference for the right hand (see Hönekopp & Watson, 2010)––the effect sizes in the present study were: left hand, $d = .38$; right hand, $d = .60$. Was the difference between the mailed-in photocopies and the ones made in the laboratory critical? Not obviously so. There was not a significant difference in mean 2D:4D between the lab and mail-ins for left or right hand or the difference between them. The correlation between 2D:4D on the left and right hands was virtually identical: .590 for the lab and .593 for the mail-ins. There was marginal evidence that for the left hands the lab photocopies resulted in sharper sex differences in 2D:4D than did the mail-in photocopies ($p = .076$ for the interaction in an analysis of variance), but this provides no compelling reason for abandoning the mail-ins.

As noted, the Australian (and Canadian) males were younger than Manning's sample, which might be a concern if the current level of androgens was at issue, but should be irrelevant to a consideration of prenatal effects (for a review concluding that prenatal organizational effects of androgens, as indexed by 2D:4D, are unrelated to adult activational effects, see Hönekopp, Bartholdt, Beier, & Liebert, 2007). In earlier papers we have argued that AR gene sequence length and finger-length ratios may each have a weak within-sex relationship, especially among women, with psychological traits for which the sexes differ, (Loehlin, Medland, & Martin, 2009; Loehlin, Medland, Montgomery, & Martin, 2005). However, this need not imply that the two are themselves correlated or causally related. Likewise, Hurd et al. (2011) reported weak but significant correlations between 2D:4D and self-reported physical aggression, and between CAG repeats and anger.

Ioannidis and Trikalinos (2005) point out, in a review of the association of genes with complex diseases, that because of capitalization on chance and publication practices "early publications may present findings that are out of proportion to the truth" (p. 543). With the present sample sizes, we obviously cannot rule out *any* association of digit ratio and the AR gene, but if one exists, it is likely considerably weaker than that initially reported by Manning, and thus less powerful in either an explanatory or predictive sense. Two failures of replication raise the question as to whether any relationship exists at all.

It should be re-emphasized that we are not here addressing the broader issue of whether prenatal androgen levels are related to individual differences in finger-length ratios, or the question of whether such a relationship is strong enough to use the latter to measure the former. We are here concerned with a more limited (but theoretically important) question: are differences in the sensitivity to androgens, as reflected in the length of the sequence of CAG repeats on the AR gene, associated with (and hence possibly responsible for) differences in finger-length ratios among individuals? Our answer is, that on present data, we see no compelling evidence of this, with one possible exception: there may be an association of CAG sequence length and right–left differences in 2D:4D-not strong enough to be statistically significant in either replication study, but at least consistent in direction.

There have been some previous studies in which right–left 2D:4D differences have been related to behavioral traits, mostly in the area of high-level athletic performance, such as fencing (Voracek, Reimer, Ertl, & Dressler, 2006) and rugby (Bennett, Manning, Cook, & Kilduff, 2011). There may also be an association with left-handedness (Beatona, Rudlinga, Kisslinga, Tourinesa, & Thomea, 2011; Manning & Peters, 2009). Whether the androgen receptor is involved in either case is not known.

In fact, few studies in the 2D:4D area have measured CAG repeats. In addition to Hurd's, Manning's, and ours, there is a recent study by Knickmeyer and her colleagues (Knickmeyer, Woolson, Hamer, Konneker, & Gilmore, 2011) in which 364 young children were studied during the first two years of life. Left- and right-hand 2D:4D, salivary levels of testosterone, and CAG repeats were measured. Most pertinent to our study, the investigators found no significant relationships between the number of CAG repeats and 2D:4D, either for the whole sample or for males and females separately. However, they did find some significant interactions in males between CAG repeats, salivary levels of testosterone, and 2D:4D, on the right hand at 1 year of age and on the left hand at 1 and 2. No significant interactions were found in females at any of the ages.

Going further afield, *complete* androgen insensitivity turns genetic males into phenotypic females, including their digit ratios (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009), and the effects of testosterone administration on cognitive empathy in women may vary with their 2D:4D ratios (van Honk et al., 2011). If this last finding is replicable (it was based on an N of 16), and if the sensitivity to testosterone was due to CAG repeats (which were not measured), and if the result generalizes to males (which is where the association between 2D:4D and CAG repeats has been reported), this study, or others like it, may be illuminating. At present, such links remain speculative, and it remains hazardous to rest theory on the assumption that there is a correlation of AR gene sequence length and finger-length ratios in the normal population.

It may be that these questions may eventually be addressable at the level of individual genes. Medland and her colleagues, in a genome-wide association study (Medland et al., 2010), reported that a particular genetic variant, LIN28B, was significantly associated with measured 2D:4D in combined British and Australian samples, an association that was successfully replicated in an additional British sample. However, direct evidence of whether this gene (or the AR gene) is causally associated with prenatal androgen levels is not presently available, and may not be easy to obtain, given the weak strengths of association typically existing between individual genes and complex traits, and the difficulties of measuring prenatal events.

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