Associations between neurochemical receptor genes, 2D:4D, impulsivity and relationship quality

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Abstract The ratio between the second and fourth digits (2D:4D) has been widely used as a proxy for fetal exposure to androgens and has been linked to a number of sociosexual traits in humans. However, the role of genes in this equation remains unknown. Here (*N*=474), we test, firstly, for associations between 2D:4D and single nucleotide polymorphisms (SNPs) in nine neurochemical receptor genes (*AR, OXTR, AVPR1A, OPRM1, DRD1/2, ANKK1, 5HTR1A/2A*), and secondly, whether digit ratios mediate the relationship between genetic variation and sociosexuality. We demonstrate significant associations between *AR*, *OPRM1* and *AVPR1A* and 2D:4D. Moreover, mediation analysis indicates that, in women, *AR* and *OPRM1* variation drives digit ratios, which are related positively to impulsivity and, for *OPRM1*, negatively to romantic relationship quality. Although these findings are subject to multiple testing issues, this study provides preliminary evidence that in women genetic factors may affect both impulsivity and perceived relationship quality through influencing factors indexed by digit ratios.

1. Introduction

The 2D:4D ratio has been widely used as a proxy for prenatal androgen levels (e.g. 1). It is sexually dimorphic (1), and correlates with sociosexual traits such as dominance (1), partner number (2), reproductive success (3), and family size (1). However, it remains unclear how the complex interaction of hormone levels and genetic expression influences both digit ratios and sociosexual phenotypes.

In mice, digit lengths are controlled by the androgen (AR) and estrogen α receptors (ER-α) (4). However, despite an initial finding, in men, that CAG-repeat variation in *AR* related to right-hand 2D:4D and the difference in 2D:4D between hands (right minus left: a measure that is generally lower in men and has been proposed as a negative indicator of androgenisation) (5,6), two meta-analyses failed to support these associations (7,8). While a recent, large-scale Genome Wide Association (GWA) meta-analysis in children and adults did find weak support for an association between 2D:4D and CAG-repeat length in females only, none of the 91 *AR* SNPs included showed significant associations (9). Although 11 loci associated with 2D:4D were identified (9), together these accounted for only 3.8% of variance in mean 2D:4D, suggesting that additional genes may also contribute.

Although (9) examined possible genetic correlations between 2D:4D and a range of diseases and physical traits, so far little has been done to investigate the relationship between digit ratios, sociosexual traits and the genes underlying them. Given that 2D:4D is often used in studies of human reproductive behavior, this knowledge is important. There is some indication that 2D:4D is linked to

DRD4 (10), a gene that is related to socially-relevant phenotypes such as aggression and impulsivity (10,11). This suggests that other genes associated with human sociosexuality may show similar relationships. If this is the case, then these genes might be either independently linked to both 2D:4D and social phenotypes, or a latent variable indexed by 2D:4D could be mediating the association between genotype and social phenotype. This paper tests between these alternatives using mediation models.

We previously reported associations between social traits, such as sociosexual orientation and romantic relationship quality, and SNP variation in the receptor genes for oxytocin (*OXTR*), vasopressin (*AVPR1a*), ß-endorphin (*OPRM1*), dopamine (*DRD1* & *2*, *ANKK1*), serotonin (*5HTR 1* & *2*) and testosterone (*AR*) (12). Here, using the same dataset, we explore (i) whether these genes show associations with 2D:4D, (ii) whether previously found relationships between these genes and sociosexuality are mediated by 2D:4D and (iii) if 2D:4D mediates associations between these genes and impulsivity.

2. Materials and methods

Healthy adults were recruited from UK science festivals and a museum. We limited the sample to Caucasians with no history of psychopathology (see 12): 474 participants (247 female, age *M*=40.2 years, range=18-75) provided both left- and right-hand digit measurements.

Hand scans were taken on a high-resolution digital scanner, palms facing downwards and rings removed. Two blind coders measured digit lengths using the Adobe Acrobat 'measure' tool. Given high inter-rater correlations (left: t_{472}=36.05, p<0.0001, R^2=0.73; right: t_{472}=35.03, p<0.0001, R^2=0.72), means for left and right were taken. We also calculated right minus left 2D:4D, Dr-l, following (5). Although it is worth noting that measurements taken directly from hands are generally larger than those from scans (e.g. 13), given that measurement was consistent across participants this should not substantially affect our results.

Participants completed the revised Sociosexual Orientation Inventory (SOI) and the Relationship Assessment Scale (RAS) (see Electronic Supplementary Material, ESM, and 12 for details). Participants also provided a saliva sample using OrageneDNA kits. The data were analysed using PLINK and SPSS, with models controlling for age and sex (see ESM for details). The full results for the genetic associations are given in Table S1 and are summarised in the main text. Sex differences in 2D:4D were generally as expected (F>M for both hands, although there was no difference in Dr-l) and men gave higher SOI scores, whereas there were no sex differences in RAS or Impulsivity (see SOM).

The optimal method for correcting for multiple tests in genetics studies remains contentious (e.g. 14). Whilst wanting to avoid false positives, the danger of false negatives is also of concern given the likelihood that complex phenotypes are polygenetic, and that each SNP will have only a small effect that is difficult to detect. Despite the issues with current correction methods, we used the

permutation mperm function in PLINK. None of the results survived this correction, indicating a need for replication studies. Current advice, however, is to interpret these results with caution.

3. Results

2D:4D and genes

The *AR* SNP rs6152 yielded a significantly positive additive effect on left (t=2.33, p=0.02), but not right, 2D:4D (Table S1). Three *OPRM1* and both *AVPR1A* SNPs also showed significant associations (Table S1). However, follow-up linear regressions split by sex demonstrated that these effects were sex-specific: Table 1. *DRD2* rs4648317 was significantly additively associated with Dr-l (Table S1), but only for females and when controlling for *domdev* effects (Table 1).

Mediation

In addition to 2D:4D, rs6152 and rs2075572 were also associated with Impulsivity, and rs2075572 and *AVPR1A* rs1117481 were also associated with RAS scores (Table S1) . Consequently, for each sex we tested whether the associations between these SNPs and Impulsivity/RAS are mediated by 2D:4D. No significant indirect effects were found. *DRD2* rs4648317 was not associated with sociosexual traits so we did not include this SNP in mediation models.

Despite the indirect effect not being significant (p=0.145), in females rs6152 was related to left-hand ratios (AA>GA>GG: t_{242}=-2.197, p=0.029: Figure 1) and

higher digit ratios were related to higher Impulsivity (t_{241}=2.141, p=0.033). No significant relationships were found for RAS or right-hand ratios.

Additionally, despite the non-significant indirect effect (p=0.110), females carrying a CC genotype for rs2075572 had higher left-hand digit ratios than G-carriers (t_{241}=2.910, p=0.004: Figure 1), and left-hand digit ratios were positively associated with Impulsivity (t_{240}=2.020, p=0.044). Similarly, higher digit ratios were related to lower relationship quality in females (t_{147}=-2.274, p=0.024; indirect effect of rs2075572 on RAS: p=0.088). Moreover, Impulsivity and RAS scores were significantly negatively related to each other independently of age (t_{152}=-2.192, p=0.030). Impulsivity was not significantly related to right-hand digit ratios.

In males, AVPR1A rs1117481 showed a significant association with right-hand 2D:4D (t_{145}=-2.917, p=0.004; Figure 2), but the latter was not related to RAS (p=0.592), so the indirect effect was not significant (p=0.617).

4. Discussion

We found that *AR* rs6152 is significantly associated with left-hand (supporting 10,15,16), but not right-hand 2D:4D (contra 5). On closer inspection, we found that this held in women only, echoing (16). Interestingly, the recent large-scale GWA meta-analysis also found weak support for an association between *AR* CAG-repeat length and 2D:4D for women only, but for both hands (9). Additionally, *OPRM1* and *AVPR1A*, two genes not directly linked to testosterone function, were significantly associated with 2D:4D. These associations were also sex-specific: genetic effects on left-hand ratios were found only in females, whereas for males right-hand ratios were affected (Table 1). The exception, rs2075572, was the only SNP associated with 2D:4D in both hands, again only in females. *DRD2* rs4648317 was found to be associated with Dr-l in women only, which chimes with recent findings of female-specific associations between Dr-l and the need for power, an implicit motivation relevant to sociosexuality (17). Moreover, this dopamine receptor gene association echoes the relationship between 2D:4D and *DRD4* found in Hadza men (10). Although intriguing, replication of these findings is needed, given firstly, that they did not survive correction for multiple comparisons, and secondly, these SNPs were not identified in the largest study to date (9). However, what is clear is that we need a stronger understanding of 2D:4D before using it as a putatively independent proxy of hormone levels in genetic studies of human sociality (e.g. 18).

In addition, we found preliminary evidence that in women, variation in *AR* rs6152 and *OPRM1* rs2075572 may indirectly affect individuals' levels of impulsivity or relationship quality, and that this effect may be mediated by

181 factors indexed by their digit ratios. According to these results, women with
182 higher (more feminine) left-hand digit ratios are more impulsive and rate their
183 romantic relationships less favourably. The direction of this relationship is
184 intriguing, since the opposite might be expected. However, recent work suggests
185 that both sexes follow one of two distinct mating strategies, one promiscuous
186 and one focusing on long-term commitment (19). If females with more feminized
187 morphology have higher 'mate value', this might be associated with
188 dissatisfaction with current partners, leading to impulsive extra-pair matings
189 and seeking alternative mates. Indeed, our results chime with higher 2D:4D in
190 women being associated with reduced delay-discounting, implying higher
191 impulsivity, which (20) link to opportunistic mating.
192
193 The association between *AVPR1a /OPRM1* and 2D:4D may reflect interactions
194 with both estrogens and androgens, which are known to regulate vasopressin
195 release and are decreased by opioids (e.g. 21,22). Given the differentiation found
196 between pairbonding behavior and *OXTR* variation in human females (23), but
197 *AVPR1A* in males (24), it is striking that the associations between *AVPR1A* and
198 2D:4D found here held in males but not females. However, right-hand digit ratios
199 were not significantly associated with relationship quality, so it remains unclear
200 this might translate into male sociosexuality.
201
202 **Ethical considerations:** The study was approved by the University of Oxford
203 Combined University Research Ethics Committee (Ref: MS-IDREC-C2-2015-005);
204 all participants provided written informed consent.
205

206 **Competing interest:** The authors declare no conflict of interest.

207

208 **Author contributions:** All authors contributed to design, data acquisition and
209 manuscript preparation. EP and RW conducted the analysis. All authors agree to
210 be accountable for the work and approve the final manuscript.

211

212 **Data** is provided as ESM.

213

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Figures

Figure 1: Left-hand 2D:4D ratios for females for different genotypes of the (A) *OPRM1* rs2075571 and (B) *AR* rs6152 SNPs. Means are shown ± 2 S.E.

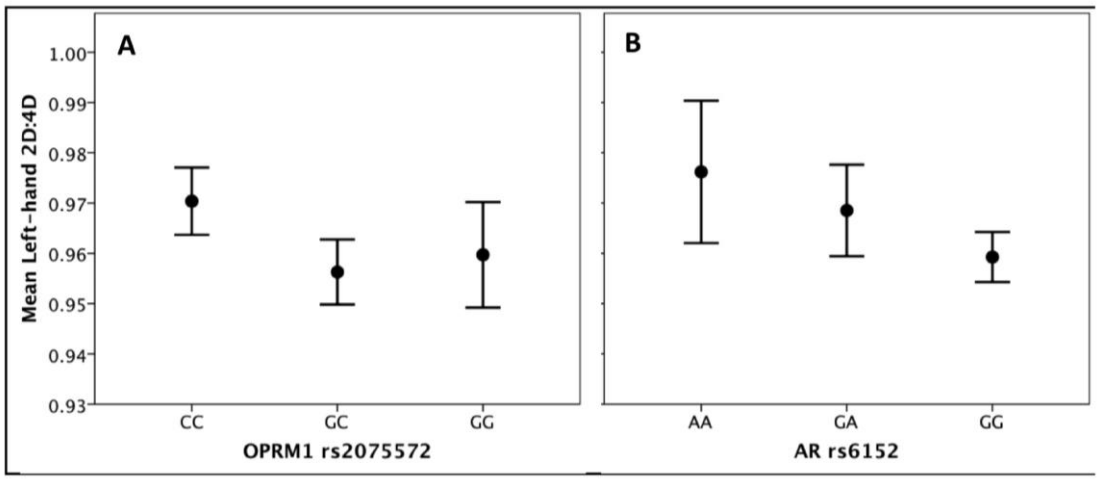
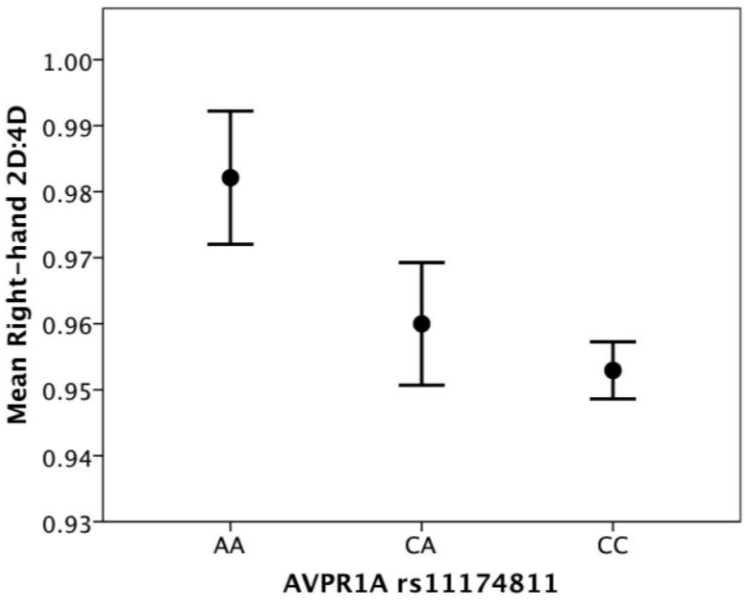


Figure 2: Right-hand 2D:4D ratios for males for different genotypes of the *AVPR1A* rs11174811 SNP. Means are shown ± 2 S.E.



319 **Table 1:** Summary of linear regressions between SNPs and 2D:4D for females
320 and males, with *add* indicating linear effects and *domdev* indicating heterozygote
321 advantage.

SNP	2D:4D	Females		Males	
		t (df)	p	t (df)	p
AR rs6152	left	*add:* -2.186 (244)	0.030	Not significant	0.653
OPRM1 rs2075572	left	*add:* 2.221 (244)	0.027	Not significant	0.115
	right	*domdev:* -2.419 (243)	0.016	Not significant	0.431
OPRM1 rs1799971	left	*add*: -1.988 (245), *domdev:* -2.403 (245)	0.048, 0.017	Not significant	0.275, 0.614
DRD2 rs4648317	Dr-l	*add: 2.040 (243), domdev: 1.401 (243)*	0.042, 0.163	Not significant	0.511, 0.533
OPRM1 rs495491	right	Not significant	0.649	*add:* -3.153 (225)	0.002
AVPR1A rs1117481	right	Not significant	0.115	*add:* -2.211 (226)	0.028
AVPR1A rs7294536	right	Not significant	0.431	*add:* -2.245 (225)	0.026

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