Androgen Receptor and Vasopressin Receptor (AVPR1a) Genetic Polymorphisms are not associated with Marital Status or Fertility among Ariaal Men of Northern Kenya

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Abstract

A growing body of scholarship implicates testosterone and vasopressin in male reproductive behavior, including in humans. Since hormones exert their effects through their respective receptors, an open question has been whether genetic polymorphisms in the androgen receptor and vasopressin 1a receptor (AVPR1a) impact human male social behavior. Here, we sought to test for associations between polymorphisms in the coding region of the androgen receptor and promoter region of AVPR1a in relation to marital status and fertility among pastoralist Ariaal men of northern Kenya. None of the three polymorphisms were related to marital status (single, monogamously married, polygynously married) or fertility (number of current living children). We discuss these null findings in light of existing data.

Key words: endocrinology, reproduction, pastoralists, hormone receptors

Introduction

There has been rapidly growing interest and progress in identifying the neuroendocrine mechanisms underlying animal social behavior (Adkins-Regan 2005). Among mammalian males, recent findings have highlighted the relevance of the steroid hormone testosterone and the peptide hormone vasopressin in the acquisition of mates and expression of paternal care. The "Challenge Hypothesis" has oriented researchers toward the role of testosterone in facilitating male mating effort (Wingfield et al. 1990), while research on vasopressin has been fueled by dramatic findings among voles demonstrating that variable forms of a receptor that binds vasopressin (the vasopressin 1a receptor, or AVPR1a) affect lab-based measures of male pair bonding and paternal care (Hammock and Young 2005).

Humans studies have increasingly integrated these nonhuman animal frameworks and empirical findings (Donaldson and Young 2008; Ellison and Gray 2009; Heinrichs and Domes 2008). A sizable and recent literature, stimulated by the "Challenge Hypothesis", finds that human males involved in long-term relationships such as marriage and/or fatherhood commonly have lower testosterone levels (reviewed in Gray and Campbell 2009). Such results have held among men in a number of North American samples, in Beijing, and among the Ariaal of northern Kenya. Many of the cognitive, mood and behavioral effects of vasopressin in humans resemble those observed in nonhuman animals, including voles (Sanchez et al. 2009). In a sample of Jamaican men engaged in paternal interactions, fathers' urinary vasopressin levels were negatively correlated with the age of their youngest child (Gray et al. 2007).

Hormones exert their effects by binding with receptors. The effects of testosterone are primarily exerted by binding with the androgen receptor, a nuclear receptor encoded on the X chromosome. Although there exist three primary vasopressin receptor types, most behavioral effects appear to occur through activation of the AVPR1a receptor. Polymorphisms in both the androgen and AVP1a receptors have been reported and implicated in differential behavioral outcomes. The polymorphisms examined here occur in the coding region of the androgen receptor and the promoter region of the AVPR1a, and in some cases physiological differences such as mRNA expression in the hippocampus have been linked with these polymorphisms (Knafo et al. 2008). Also of interest, testosterone is capable of modulating vasopressin levels and vasopressin receptors, indicating that these hormones exhibit synergies that may facilitate successful male reproductive behavior.

In humans, most investigations of androgen receptor polymorphisms have centered on variation in CAG repeats in the coding region (reviewed in Zitzmann and Nieschlag 2003). For example, men in India convicted of rape or murder had fewer CAG repeats than control men (Rajender et al. 2008). Most AVPR1a human research has focused on two regulatory areas referred to as RS1 and RS3 (reviewed in Israel et al. 2008). More repeats (longer alleles) in the RS3 have been variably related to play in experimental economics games, prepulse inhibition, and other RS3 alleles linked to creative dance and music. In a Swedish study, the 334 allele of the RS3 region was associated in men with lower scores on a partner bonding scale, lower likelihood of being married, and lower partner assessments of "affectional expression" (Walum et al. 2008).

Here, we sought to test whether androgen receptor and AVPR1a polymorphisms

would be associated with male marital status and fertility among the Ariaal, pastoralists of northern Kenya. The existing human testosterone and vasopressin findings are limited in their geographic scope, and neglect subsistence populations like the Ariaal.

Methods

Field research was conducted among the Ariaal, pastoralists of northern Kenya. Details concerning the study population, sample, and field protocol have been reported in Campbell et al. (in press). Of interest here, the Ariaal exhibit polygyny and do not use contraception, a very different cultural context from previous studies. Methods for obtaining head hair samples and genotyping the androgen receptor promoter CAG repeats are also reported in Campbell et al. (in press).

The AVPR1a was typed for two promoter microsatellites, RS1 and RS3. The RS1 PCR reaction contained 1.25 µM forward primer (AGGGACTGGTTCTACAATCTGC), 1.25 µM reverse (ACCTCTCAAGTTATGTTGGTGG), 2.5 mM dNTP, 2.5 mM $MgCl_2$, 0.625 units AmpliTaq Gold (ABI), 1X Buffer (ABI), 3 µl DNA template in a total volume of 25 µl. The PCR product ranged from 304-336 bp in length. Repeat number was calculated by subtracting 300 and then dividing by 4. The RS3 PCR reaction was identical to the RS1 except the primers were TCCTGTAGAGATGTAAGTGC-forward and TCTGGAAGAGACTTAGATGG-reverse The RS3 PCR product ranged from 315-349 bp in length. Primers for AVPR1a were fluorescently labeled. Cycle conditions were: 8 min at 95°C, 40 cycles of 30 s at 95°, 30 s at 55°, 1 min at 72° and one final extension of 7 minutes at 72°. Products were analyzed on an ABI 3100 genetic analyzer using GeneMapper 3.7 (ABI). We experienced some difficulties in distinguishing between

adjacent alleles (2 bp differences) at the RS3 polymorphism that we could not resolve with multiple PCR or analyzer runs. As a result, there are likely some errors ±1 repeat unit in the genotypes presented here, which likely makes our tests more conservative with respect to finding an association between RS3 alleles and phenotypes. For comparison purposes, we note that the PCR products amplified by our primers contain 7 bp more flanking sequence than Walum et al. (2008).

For statistical tests, we employed ANCOVA, with marital status (0, 1, or 2+ wives) and fertility (number of reported living kids) as dependent variables and age group, residence, and fat free mass as control variables. For androgen receptor analyses, we both treated CAG repeats as a continuous variable and used a dichotomous split of repeats at 20 (like in Campbell et al. in press). For AVPR1a analyses, we followed recent methods of dichotomizing alleles in high/low by a median split for both the RS1 and RS3, with subjects then categorized as having 0, 1, or 2 high repeat alleles.

Results

As documented in other human studies, we observed polymorphisms in the androgen and AVPR1a receptors among the Ariaal (Table 1). However, neither these androgen receptor polymorphisms nor AVPR1a polymorphisms predicted marital status or fertility among Ariaal men in the sample (all $p>0.05$). There were no significant interactions between genotypes and residency either. In post hoc analyses, the addition of morning or afternoon testosterone levels did not alter the null direct effects or interactions involving androgen receptor polymorphisms. Further, post hoc analyses testing for effects of AVPR1a alleles at >15% frequency at both RS1 and RS3 regions also did not alter the

null findings. The presence of our 341 allele (equivalent to Walum et al.'s 334 allele) was also unrelated to marital status or fertility.

Discussion

The behavioral, neurological, and cognitive significance of marriage and fertility vary cross-culturally. Unlike the Swedish population, the Ariaal practice polygyny and do not use contraception, which may help to account for our null findings. Furthermore, our sample size was sufficient to detect an effect size of $d = 0.5$ at 80% power and two-tailed p; the effect sizes of the associations between existing receptor polymorphisms and human behavioral measures vary (e.g., $d = 0.5$ in an experimental economics and AVPR1a study [Knafo et al., 2008] and $d = 0.27$ for effects on partner bonding in the Swedish study). We should thus not expect to find large, robust relationships between complex traits measured in "naturalistic" samples and genetic variants. Still, further research better characterizing the physiological, cognitive, and behavioral effects of polymorphisms like those investigated here bridges the experimental animal literature with human data "on the ground", in turn potentially revealing the more subtle, additive effects of genes to complex, polygenic traits.

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Table 1. Hormone Receptor Genetic Polymorphism Frequencies

Androgen Receptor CAG Repeats			AVPR1a RS1 Alleles			AVPR1a RS3 Alleles	
Repeat #	Frequency		Allele	Frequency		Allele	Frequency
15	1		1	6		315	3
16	3		2	21		317	1
17	0		3	97		319	0
18	19		4	95		321	9
19	14		5	16		323	12
20	14		6	49		325	42
21	19		7	18		327	30
22	12		8	9		329	44
23	22		9	1		331	33
24	22					333	9
25	13					335	5
26	16					337	38
27	1					339	41
28	5					341	27
29	0					343	4
30	4					345	1
31	0					347	0
32	0					349	3
33	0						
34	1						

Note that subjects have two copies of the AVPR1a alleles and one copy of the AR. Also note that given the primers we used our AVPR1a RS3 341 allele corresponds with the 334 allele identified in Walun et al. (2008).