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Courtney A. Ficks Date

Vasopressin and social behavior in humans: testing for genetic associations using a latent factor approach

By

Courtney A. Ficks
Doctor of Philosophy

Psychology

_____
Irwin D. Waldman, Ph.D.
Advisor

_____
Patricia Brennan, Ph.D.
Committee Member

_____
Jocelyne Bachevalier, Ph.D.
Committee Member

_____
Scott Lilienfeld, Ph.D.
Committee Member

_____
Larry Young, Ph.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

Vasopressin and social behavior in humans: testing for genetic associations using a latent factor approach

By

Courtney A. Ficks
B.S., Arizona State University, 2007
M.A., Emory University, 2010

Advisor: Irwin D. Waldman, Ph.D.

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Abstract

Vasopressin and social behavior in humans: testing for genetic associations using a latent factor approach

By Courtney A. Ficks

Arginine vasopressin (AVP) is a neuropeptide that shows strong evolutionary conservation across species and has been demonstrated to play a role in a wide range of social behaviors. Several candidate gene studies have reported associations between the AVPR1a receptor gene, *AVPR1A*, and variation in social phenotypes in humans. Nonetheless, as studies of other candidate genes have also demonstrated, the magnitude and direction of effects for *AVPR1A* polymorphisms have been inconsistent and difficult to replicate, perhaps due to factors such as multiple testing and differences in linkage disequilibrium of SNPs between study samples. In the present dissertation we explored a method of testing for the effects of *AVPR1A* that allowed us to examine the effects of common variation across the gene using a single omnibus statistical test. Our sample included 621 children ages 6-18 years who were initially recruited from several sources as part of an ongoing study of the etiology of common childhood psychiatric disorders, behavior problems, and temperament and personality. Children were genotyped for 8 single nucleotide polymorphisms (SNPs) in *AVPR1A* that were selected a priori to cover the majority (> 80%) of the variation across the gene. Parents provided reports of their children's behavior and temperament. Following a rigorous examination of genotyping quality, we tested for associations between *AVPR1A* and two social phenotypes (aggression and sociability) in analogous sets of structural equation models. In each model, the phenotype was modeled as a latent factor indicated by the parent-reported items for the scale of interest. Similarly, *AVPR1A* genotype was modeled as a separate latent factor indicated by the categorically-coded genotypes for each of the 8 SNPs. The latent phenotype factor was regressed on several covariates (i.e., age, sex, and ethnicity) as well as the latent *AVPR1A* factor. Findings for these

models demonstrated significant associations between *AVPR1A* and childhood aggression for both boys and girls. Associations were fairly robust to differences in sample size and the number of observations available per SNP. In contrast, *AVPR1A* was not associated with sociability. Overall, the proposed gene-based, latent factor approach to modeling genetic associations may facilitate more powerful and replicable tests of association for the effects of *AVPR1A* on social behavior than typical analytic methods practiced in candidate gene research.

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General Introduction

Arginine vasopressin (AVP) is a peptide that has shown strong evolutionary conservation across mammalian species (Caldwell & Young, 2006) and appears in analogous forms in most vertebrates (Gimpl & Fahrenholz, 2001). Decades of animal and human research has uncovered numerous roles for this peptide, which influences blood pressure, water reabsorption, and myometrial contraction processes within the peripheral nervous system (Martin, 2012; Thornton et al., 2002). Yet the focus of the current investigation is not on the peripheral actions of AVP, but on the surprisingly complex actions that this peptide—or "social neuropeptide" as it is sometimes characterized (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011)—has within the central nervous system (CNS). Indeed, AVP appears to contribute to a wide range of complex social behaviors, including parenting behaviors, sexual behavior, the formation of social bonds, aggression (Caldwell & Young, 2006), and social recognition and communication (Albers, 2012; Bielsky & Young, 2004).

Differences in these types of social behavior across species have been attributed, at least in part, to variation within CNS AVP systems. Nonetheless, it remains uncertain to what extent differences in social behavior within the human species may also be attributed to these systems. Polymorphisms have been identified within AVP-ergic system genes, and there is some evidence to suggest that these polymorphisms influence variation in social behavior, including psychopathology characterized by social deficits in both human and nonhuman animals (Hammock, Lim, Nair, & Young, 2005). Yet candidate gene studies of complex traits such as these have been the subject of considerable criticism in recent years for their small effect sizes and failure to yield replicable results (Duncan & Keller, 2011; Hewitt, 2012). Consequently, more robust evidence is needed to determine whether genetically-influenced variation in AVP levels contributes to intraspecies variation in social behavior. In the following studies, we tested for

associations between a commonly studied AVP-ergic gene and two forms of social behavior—aggression and sociability—using a novel analytic method designed to better reflect the variation across this gene and its putative impact on social phenotypes.

Vasopressin in the Human Body

AVP comprises an oligopeptide chain of nine amino acids (Gimpl & Fahrenholz, 2001) coded from *AVP*, a gene located on chromosome 20 in humans (Gimpl & Fahrenholz, 2001). AVP is a full agonist for several classes of AVP-ergic receptors, including AVPR1a, AVPR1b, and AVPR2 receptors, and also serves as a partial agonist for oxytocinergic (OT) receptors (Sharman et al., 2011). AVP-ergic receptors are located in peripheral regions including the liver, heart, and kidneys (Thibonnier et al., 1996), adrenal glands (Grazzini et al., 1999), and myometrium (Thornton et al., 2002). In addition, AVPR1a and AVPR1b (but not AVPR2) receptors are expressed throughout the brain, within the frontal cortex, olfactory system, hypothalamic nuclei, and cerebellum, among other regions (Hernando, Schoots, Lolait, & Burbach, 2001; Szot, Bale, & Dorsa, 1994).

Vasopressin in Social Behavior

Several avenues of investigation within the extant literature have yielded broad, convergent evidence for the role of vasopressin in social behavior. These studies have utilized cross-species comparative approaches, experimentation via pharmacological or genetic manipulation, and correlational methods within species. We will focus briefly on cross-species comparison before taking a more in-depth look into experimental research, which comprises the majority of this literature. Finally, we will discuss association studies in humans, including candidate gene research, and proceed with implications for the current investigation.

Cross-Species Comparison

Comparative approaches have been used to examine structural and functional differences in AVP expression in the CNS across species that differ in their patterns of social bonding. Most notably, neuroanatomical comparisons between various species of vole, which have been shown to differ in the extent to which they form monogamous (exclusive) social bonds (e.g., Carter & Getz, 1993; Getz, Carter, & Gavish, 1981), have revealed significant differences among monogamous and polygamous (non-exclusive) species. Specifically, although patterns of AVP neuronal distribution have appeared similar between species (Young, Gobrogge, Liu, & Wang, 2011), patterns of receptor density across various brain regions have differed for monogamous *prairie* voles and their polygamous *montane* counterparts. Specifically, prairie voles exhibited higher densities of AVP receptors across various forebrain and midbrain structures (including but not limited to the diagonal band, bed nucleus of the stria terminalis [BNST], cingulate cortex, central nucleus of the amygdala, and several regions of the thalamus) and lower densities of AVP receptors in the lateral septum, ventral subiculum, and in the brainstem and cerebellum (Insel, Wang, & Ferris, 1994). These differences in AVP expression are apparent at birth, and following transient changes in expression over the first three weeks of life, appear to remain consistent across the remainder of the lifespan (Wang, Liu, Young, & Insel, 1997).

The apparent neuroanatomical differences in AVP expression among species varying in social behavior have since inspired a plethora of experimental research into the putative functional role of AVP in the formation of social bonds as well as other forms of social behavior (which will subsequently be discussed). Nonetheless, it is important to note that cross-species comparative approaches have been criticized for underutilizing taxonomic diversity and an inability to explain natural variation *within* species (Ophir, Wolff, & Phelps, 2008; Phelps, Campbell, Zheng, & Ophir, 2010). Greater breadth in the monogamous and polygamous species

sampled and attention to natural within-species variation in statistical analysis may allow us to infer the underlying causes for these reported phenomena with greater confidence.

Experimental Evidence

Laboratory investigations into the putative functional roles of AVP systems in social behavior within species, including both affiliation and aggression, have used pharmacological manipulation (primarily via AVP receptor agonism and/or antagonism) as well as genetic manipulation in order to demonstrate the importance of this neuropeptide in various social behaviors. Of these behaviors, those with the most direct relevance to the current investigation include social recognition, social bonding, and aggression.

Social recognition in nonhuman animals.

Following early evidence for a role of AVP in learning and memory (van Wimersma Greidanus, Van Ree, & De Weid, 1986), investigators found that subcutaneous and intraventricular injections of AVP facilitated the recognition of recently encountered juvenile conspecifics (as indicated by exploration time during the subsequent encounters) in adult male rats (Le Moal, Dantzer, Michaud, & Koob, 1987; Sekiguchi, Wolterink, & van Ree, 1991), whereas injections of dPTyr(Me)AVP (an AVPr1a receptor antagonist) appeared to have inhibitory effects on recognition (Dantzer, Bluthe, Koob, & Le Moal, 1987). Follow-up studies indicated that AVP-ergic effects on social recognition were at least partially mediated by innervation of the lateral septum (Bychowski, Mena, & Auger, 2013; Dantzer, Koob, Bluthe, & Le Moal, 1988; Engelmann & Landgraf, 1994; Everts & Koolhaas, 1997) and both AVP-ergic and noradrenergic neurons within the olfactory bulb (Dluzen, Muraoka, Engelmann, & Landgraf, 1998; Dluzen, Muraoka, & Landgraf, 1998; Tobin et al., 2010) and appeared to operate independently of peripheral AVP-ergic endocrine activity (Popik, Wolterink, De Brabander, & van Ree, 1991).

To some extent, it appears that these AVP-ergic influences on social recognition are sexually dimorphic. Although female rats have shown improvement in social recognition tasks following AVP administration, AVP receptor antagonism has not yielded the same deficits in female social recognition as it has in male animals (Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012). In addition, the actions of vasopressin on social recognition are facilitated by androgen expression in males. Intact but not castrated male rats and mice showed a decline in social recognition following peripheral administration of dPTyr(Me)AVP (Bluthe, Gheusi, & Dantzer, 1993; Bluthe, Schoenen, & Dantzer, 1990), and furthermore, the implantation of testosterone capsules resulted in the emergence of these effects in the castrated rats (Bluthe et al., 1990). Lesioning the vomeronasal organ, which is involved in olfactory chemoceptive social communication in male rats and projects to the accessory olfactory bulb, resulted in patterns of AVP antagonist-insensitivity similar to that of castrated males, indicating that this structure may be involved in an androgen-dependent AVP-ergic social recognition pathway, at least in males (Bluthe & Dantzer, 1993).

More recently, genetic knockout mice lacking functional copies of the AVPR1a receptor gene (*AVPR1A*) also exhibited impaired social recognition (Bielsky, Hu, Szegda, Westphal, & Young, 2004), a deficit which did not appear to be due to differences in non-social learning impairment (Bielsky et al., 2004; Bielsky & Young, 2004) and which could be reversed via re-insertion of *AVPR1A* in the lateral septum (but not the medial amygdala) using a viral vector (Bielsky, Hu, Ren, Terwilliger, & Young, 2005). Although *AVPR1B* knockout mice also displayed deficits in social recognition, these effects appeared to be less pronounced (Wersinger, Ginns, O'Carroll, Lolait, & Young, 2002).

Overall, it appears that AVP plays a role in social recognition in both rats and mice. Crucial structures within AVP-ergic pathways involved in social recognition include the

vomeronasal organ, olfactory bulb, and lateral septum, and it appears that both norepinephrine and androgens serve to modulate AVP's influence within these pathways. Nonetheless, more research is needed to better understand sex differences in these underlying neuronal pathways, including the putative role(s) of gonadal hormones.

Social recognition in humans.

There is little experimental research examining the role of AVP in human social recognition. Our knowledge of the effects of this neuropeptide on human social recognition has come from only a few studies published within the last several years, which merits discussion of these findings in greater depth.

In an examination of the effects of intranasal AVP on facial recognition, Guastella, Kenyon, Alvares, Carson, and Hickie (2010) conducted a double-blind, randomized trial in which 48 male participants received administrations of either AVP or a placebo intranasally and were asked to view an array of 54 faces displaying happy, neutral, or angry expressions. The following day, participants completed a "surprise memory test" in which they viewed the same 54 faces in addition to 54 novel faces (108 total) in a randomized order. For the test, participants were asked to rate (by pressing separate computer keys) to what extent they recognized each face: *remember* ("they could recollect specific details about a face from the study session"), *know* ("the face felt familiar but they could not recollect specific details"), or *new* ("they believed the face was not presented at study"). Planned contrasts revealed a significant drug X valence interaction in which participants receiving AVP were more likely to *know* happy and angry faces when compared to neutral faces (Guastella et al., 2010), and it appeared that this finding was driven by accurate *know* judgments. These findings implied that increased AVP improved human recognition for emotionally-valenced faces, but a greater wealth of evidence is needed to

determine whether these findings are replicable, and if so, to explain what mechanisms underlie these putative effects.

In an effort to better understand the neurobiological action of AVP administration on cognition in humans, Zink et al. (2011) administered intranasal AVP (or placebo) to 20 healthy adult males approximately 45 minutes prior to the completion of a task that involved matching negatively valenced faces or scenes. Stimuli to be matched were either familiar (seen during a "training run" one week prior) or novel. Both participant groups performed well at the task and showed faster reaction times when matching familiar stimuli, but there were no improvements in performance in those who had received intranasal AVP when compared to those who had received the placebo (Zink et al., 2011). Although AVP did not appear to improve recognition, accuracy at matching neared 100% across both patient groups, and it is thus possible that ceiling effects in task performance may have masked any differences between groups. Investigators did find significant differences in neural activation between AVP-induced participants and controls. Specifically, activity in the left TPJ/Brodmann area was increased for unfamiliar faces and scenes in participants who had received the placebo, whereas this effect was absent in those who had received intranasal AVP (Zink et al., 2011). At this time, it is unclear whether AVP receptors are present within this region, though previous research has implicated the TPJ in social recognition and theory of mind (see Zink et al., 2011 for further discussion). No differences in activation in the lateral septum were found between groups, and it is thus unclear whether this region plays a similar role in human social recognition as has been found in other species. Clearly, more research is needed to determine whether these neuropeptides influence human social recognition on a basic level, and to whether any potential effects are short- versus long-term in nature. Although there is some initial evidence for alterations in human facial recognition following AVP administration, these effects have yet to be replicated.

Social bonding in nonhuman animals.

There is a wealth of experimental evidence supporting a role for AVP in the formation of social bonds, and multiple reviews are available on the topic (Insel, 2010; Lim & Young, 2006; Young et al., 2011). The most widely studied type of social bond with regard to AVP has included the pair bond, which occurs between mated individuals within a monogamous species (see previous discussion entitled Cross-Species Comparison). Although pair bonding is not as relevant to the current investigation as general affiliative behavior, the literature on the effects of vasopressin on pair bonding is the most fully developed and may provide insight into the mechanisms underlying general social affiliation.

Laboratory studies of prairie voles (previously discussed), which serve as a rodent model of human monogamy, have shown that both males and females in this species develop preferences for spending time with specific, familiar partners with whom they have previously spent time cohabiting or mating over unfamiliar partners (Young et al., 2011). To some extent, the neurobiological mechanisms underlying this process appear to be sexually dimorphic (Insel & Hulihan, 1995), and AVP's role in the development of partner preference has been most evident in males. Both intraventricular injection of AVP (Winslow, Hastings, Carter, Harbaugh, & Insel, 1993) and experimentally increasing AVPr1a receptor binding in the ventral pallidum (Pitkow et al., 2001) facilitated partner preference in male prairie voles, and administering an AVPr1a antagonist intraventricularly or into the ventral pallidum (or reducing pallidal AVPR1a receptor density through injection of a viral vector) blocked partner preference formation in males (Barrett et al., 2013; Lim & Young, 2006; Winslow et al., 1993) but not in females (Insel & Hulihan, 1995).

Experimental findings have also elicited several of the mediational processes involved in AVP's effects on pair bonding. The olfactory system, previously discussed for its role in social

recognition, also appears to be important for pair bonding in voles. Lesioning the vomeronasal organ (Curtis, Liu, & Wang, 2001) or removing the olfactory bulb (Williams, Slotnick, Kirkpatrick, & Carter, 1992) inhibited partner preference formation in females. In addition, the dopaminergic system, which has been shown to play a role in addiction and habit formation (Berke & Hyman, 2000), has been shown to impact partner preference formation in both male and female voles. Activation of dopaminergic D2 receptors in the nucleus accumbens of females via quinpirole induced partner preference formation following brief (6 hour) cohabitation without mating, whereas bilateral antagonism of D2 receptors via eticlopride in this region inhibited partner preference formation following 24 hours of cohabitation and mating (Gingrich, Liu, Cascio, Wang, & Insel, 2000). Similarly, intraperitoneal injections of apomorphine (a non-selective dopamine agonist) induced partner preference in males following 6 hour cohabitation whereas haloperidol (a nonselective dopamine antagonist) blocked partner preference following 24 hours of cohabitation and mating (Aragona, Liu, Curtis, Stephan, & Wang, 2003), indicating that dopamine may play a role in pair bond formation in both sexes.

In addition to pair bonding, experimental research with nonhuman animals has also examined bonds between mothers and infants. Briefly, these studies have utilized the traditional rat and mouse models as well as the more unique sheep model (as sheep, like humans, have been shown to be more discriminating in the offspring to whom they deliver maternal care Debiec, 2007; Insel, 2010; Lim & Young, 2006). Bosch and Neumann (2008) found that intracerebroventricular infusions of AVP and upregulation of AVP receptors in the medial preoptic area increased the frequency of maternal behaviors in rats (specifically arched-back nursing), whereas AVPr1a antagonism or downregulation of AVP receptors in the medial preoptic area decreased maternal behaviors (arched-back nursing and time spent in direct contact with pups) (Bosch & Neumann, 2008). In another study, Bosch, Pfortsch, Beiderbeck,

Landgraf, and Neumann (2010) reported that lactating rats showed increased AVPr1a binding in the bed nucleus of the stria terminalis and increased AVP release in the medial preoptic area during interactions with pups (Bosch et al., 2010). AVPr1a antagonism in the bed nucleus of the stria terminalis did not alter maternal behavior (e.g. nursing, pup retrieval) but reduced maternal aggression toward a female intruder (Bosch et al., 2010), indicating that the respective effects of these neuropeptides on maternal behavior may be complex and/or behavior-specific. The role of these neuropeptides in aggression will be discussed in greater detail in subsequent sections.

Social bonds and relationships in humans.

Although the nonhuman literature has primarily focused on the role of AVP in pair bonding and maternal care, human experimental research (which is limited) has primarily emphasized the role of this neuropeptide in social information processing. Based on these studies, it appears that AVP may play a role in the processing of emotionally-valenced information, with some findings suggesting that its effects differ for males and females. Thompson, Gupta, Miller, Mills, and Orr (2004) examined the effects of intranasal AVP on facial EMG activity and several indicators of autonomic arousal (heart rate, blood pressure, and skin conductance) in a sample of male college students (Thompson et al., 2004). In this study, participants viewed a series of 18 angry, neutral, and happy facial images on a computer screen for 8 seconds per face while completing a color-matching task. Investigators reported increased corrugator EMG responses (associated with anger/threat) to neutral, but not happy or angry faces. No differences in autonomic arousal were found between groups (Thompson et al., 2004).

In a follow-up study, the investigators used similar methodology in testing both male and female college students' responses to facial emotion stimuli after intranasal AVP or placebo administration (Thompson, George, Walton, Orr, & Benson, 2006). Participants viewed 2 sets of

27 same-sex faces (displaying neutral, angry, or happy emotions) projected onto a screen 15 minutes and 50 minutes, respectively, following drug administration. Facial EMG activity, skin conductance, heart rate, perceptual ratings regarding participants' opinions of the "approachability/friendliness," and self-reported state anxiety following the task were recorded. As in the previous study, male participants who received AVP showed stronger corrugator EMG responses to neutral faces 15 minutes following administration. In addition, males receiving AVP rated happy faces as significantly less approachable than control males. In contrast, women who received AVP showed stronger zygomaticus (smiling) responses to neutral faces 50 minutes after administration and rated neutral faces as significantly more approachable than control women. In the full sample, AVP also increased anxiety following the task and increased skin conductance to angry and neutral faces, but no additional analyses were conducted to determine whether these effects differed by sex (Thompson et al., 2006). Thus, it appears that males receiving AVP may have lower thresholds for detecting threat in facial expressions, whereas for females the opposite may be true. This finding coincides with another recent finding that AVP administration increased the responsiveness of males (but not females) with post-traumatic stress disorder (PTSD) to their romantic partners' expressions of anger (Marshall, 2013).

On a similar note, in a study that recently utilized the Reading of the Mind in the Eyes Test, male participants who received AVP made more mistakes in recognizing "negative" emotions than controls males, but only for same-sex individuals. This deficit was not apparent in recognizing "positive emotions." However, based on the findings reported, it is unclear what types of errors were made (i.e. whether differing negative emotions were perceived as interchangeable or whether systematic biases were present) and thus it is difficult to determine whether these findings are in agreement with previous findings for AVP and social cognition (Uzefovsky, Shalev, Israel, Knafo, & Ebstein, 2012).

Although most studies have examined the role of AVP in social information processing using visual imagery, there is some evidence to suggest that these findings may also be applicable to the processing of social information through the auditory system. Guastella, Kenyon, Unkelbach, Alvares, and Hickie (2011) examined whether AVP administration affected the processing of sexual words (Guastella et al., 2011). Male participants responded to various categories of words (including words describing relationships, sex, and safety as well as other words) by selecting the word's valence (positive or negative) as quickly as possible. The investigators reported that although both groups performed similarly in terms of correct responses, there was a word type X treatment interaction in that males who received AVP showed significantly faster response times for sexual words versus other word categories (Guastella et al., 2011). Thus, it appeared that AVP specifically facilitated the processing of sexual information. Further research is needed to determine whether these effects may be replicated and/or generalized to visual imagery or other types of socially-relevant auditory cues. Further, more information regarding the role of AVP administration on female social information processing is needed before we may infer whether these effects may be generalized to females.

To some extent, AVP may impact individual behavior through stimuli-dependent activation of the autonomic nervous system. Shalev et al. (2011) reported that AVP administration in males increased both salivary cortisol and heart rate in response to tasks involving social evaluation but had no effect on cortisol levels or heart rate across several other types of tasks that did not include a social evaluative component (Shalev et al., 2011). In addition, Thompson et al. (2006) postulated that the previously discussed finding of sex differences in the effects of AVP administration on males' and females' facial responses to neutral facial stimuli may have been due to sex differences in the autonomic response to social

threats; in addition to the typical "fight or flight" response to environmental threats, females may to some extent utilize a "tend and befriend" response, which would explain why females exhibited stronger "smiling" responses to neutral faces (Thompson et al., 2006). Autonomic effects may depend on alterations in patterns of neural connectivity in the brain, as male participants who received intranasal AVP prior to an fMRI matching task (containing fearful/angry faces as well as non-facial objects) showed greater activity in the medial prefrontal cortex than controls when matching fearful/angry faces (Zink, Stein, Kempf, Hakimi, & Meyer-Lindenberg, 2010), and structural equation modeling suggested AVP-ergic effects on connectivity between the subgenual and supragenual cingulate in both hemispheres, regions which have been previously implicated in the regulation of fear (Zink et al., 2010). Additional discussion regarding the role of AVP in response to threat, including aggression, will be discussed in subsequent sections.

Two recent studies went beyond examining the effects of AVP administration on social information processing to examine the effects of this neuropeptide on social behavior (Rilling et al., 2013; Rilling et al., 2012). In the first study, Rilling et al. (2012) used a variation of the classic Prisoner's Dilemma paradigm to examine whether AVP administration affected individuals' tendencies for partner cooperation versus defection in a sample of males. In the second study, this paradigm was repeated with a sample of females (Rilling et al., 2013). Participants in these studies completed four rounds of the paradigm in an MRI scanner while partnered with a computer algorithm designed to mimic human playing strategies. During each round of the game, Player 1 (either the participant or the computer) made their choice to cooperate or defect (D) first, and then Player 2 made a decision given knowledge of Player 1's choice. Choices were rewarded as follows: CC = $2/$2, CD = $0/$3, DC = $3/$0, and DD = $1/$1 for Players 1 and

2, respectively. Participants completed the task as Player 1 for 2 rounds and Player 2 for 2 rounds.

In the sample of men, AVP administration resulted in significant increases in reciprocated cooperation (i.e., cooperating following Player 1's cooperation) (Rilling et al., 2012). In the sample of women, AVP administration did not appear to affect reciprocated cooperation, but instead it affected conciliatory behavior; participants were more likely to cooperate following their partner's defection (Rilling et al., 2013). The reciprocated cooperation seen in the male sample was associated with increased activation in several neural regions, including the BNST, the lateral septum, and the stria terminalis, as well as increased amygdalar connectivity with the subgenual anterior cingulate cortex (Rilling et al., 2012). No such increased activation was seen in the female sample (Rilling et al., 2013), suggesting the impact of AVP administration on neural activation may be sexually dimorphic. Overall, the sex-specific effects of this neuropeptide on the behavioral and physiological components of social decision-making may not be surprising given previous reports that AVP seems to promote differential patterns of threat response in men and women (i.e., "fight or flight" vs. "tend and befriend"), but a greater number of studies with corroborating evidence will be needed to support such claims. This topic will be discussed further in the introduction to Study 2.

Social dominance and aggression in nonhuman animals.

There has been extensive research on the role of AVP in social dominance and aggression in nonhuman animals. Early studies examined the roles of AVP and vasopressinergic analogs (e.g. lysine vasopressin) in social hierarchies among rodents. Investigators reported that these compounds enhanced the effects of social defeat on future submissiveness and attack avoidance in male mice (Leshner & Roche, 1977; Roche & Leshner, 1979), but only when administered *following* social defeat (Siegfried, Frischknecht, & Waser, 1984). In contrast, when

vasopressin was administered *prior* to defeat, it actually appeared to interfere with the acquisition of the avoidant/submissive response (Siegfried et al., 1984).

In addition, studies utilizing golden hamsters, which have been shown to communicate dominance with other males through "flank marking" (territorial scent marking), demonstrated that social dominance patterns could be altered through AVP-ergic manipulation (Ferris, Meenan, Axelson, & Albers, 1986). Specifically, when "subordinate" hamsters within male hamster pairs were treated with AVP (via anterior hypothalamus-medial preoptic area microinjection), they showed drastic increases in flank marking behavior, although it appeared that the untreated "dominant" hamsters within the pairs did not alter their own marking habits (Ferris et al., 1986). In contrast, "dominant" hamsters treated with an AVP-ergic antagonist showed drastic reductions in flank marking , which in turn resulted in increased marking behavior among their untreated "subordinate" housemates (Ferris et al., 1986). Interestingly, all treatment effects were temporary; following cessation of AVP and antagonist administration, all animals returned to their original patterns of social dominance (Ferris et al., 1986). From these findings, it appears that AVP plays a significant role in behavior related to social dominance and submission.

In addition to affecting established social dominance structures, AVP appears to play a direct role in aggressive behavior. AVP-ergic antagonism in the anterior hypothalamus reduced hamsters' aggressive behavior ("biting attacks") toward intruders (Ferris & Potegal, 1988) and an orally administered APV1b antagonist significantly reduced chasing behavior and offensive sideways attacks in addition to flank marking (Blanchard et al., 2005). These effects were present as early as puberty; juvenile male hamsters who received anterior hypothalamic microinjections of an AVPr1a receptor antagonist showed greater latencies to attack, fewer attacks, and fewer bites in offensive play fighting than untreated controls (Cheng & Delville,

2009). In addition, there was some indication that the effects of AVP may be limited to aggression in the context of territorial/hierarchical disputes, as AVP1b knockout mice exhibited less offensive intermale aggressive behavior than wild-type littermates (Wersinger, Caldwell, Christiansen, & Young, 2007; Wersinger et al., 2004) but displayed otherwise intact levels of self-preservation (including defensive avoidance and aggressive response to food deprivation) (Wersinger et al., 2007).

AVP-ergic analogs have also been shown to affect aggressive behavior in non-rodent species, including pecks, beak fences, and/or chases during mate competition in zebra finches and violet-eared waxbills (Goodson & Adkins-Regan, 1999; Goodson, Kabelik, & Schrock, 2009) and territorial behavior in both the bluehead wrasse (Semsar, Kandel, & Godwin, 2001) and the Amargosa River pupfish (Lema & Nevitt, 2004). As in rodents, vasopressinergic effects in other species appear to be at least partially dependent upon context and social status (e.g., Ferris et al., 1986; Semsar et al., 2001). This contextual dependence may be a product of environmentally-induced structural changes within the AVP system; social isolation, which increased the frequency and duration of aggression in Syrian hamsters, also increased AVPr1a receptor binding in the paraventricular nucleus and anterior and lateral hypothalamus and decreased binding in the central amygdala (Albers, Dean, Karom, Smith, & Huhman, 2006). Consequently, associations between social experience, social behavior, and AVP may be multidirectional.

Although much research on the effects of AVP on aggression has utilized male subjects, there is some evidence for its effects in females. In contrast to findings reported in males, female Syrian hamsters receiving anterior hypothalamic injections of an AVPr1a antagonist displayed *increased* aggressive behavior (as indicated by latency to attack and duration of aggression) in a dose-dependent manner, whereas injection of AVP *reduced* aggressive behavior

(Gutzler, Karom, Erwin, & Albers, 2010), in other words exhibiting behavioral effects opposite of those typically seen in males. Similarly, intracerebroventricular injection of AVPr1a antagonists in primiparous lactating female rats increased aggressive behaviors (indexed by number of attacks, attack duration, and latency) toward male intruders; AVP administration increased latency to attacks but otherwise did not affect the females' aggressive behavior (Nephew & Bridges, 2008). These effects were replicated in another sample of multiparous female rats (described as displaying higher mean levels of aggression), with AVP infusion in this study also leading to significant reduction in attack duration (Nephew, Byrnes, & Bridges, 2010). Interestingly, it appeared that the effects of AVP were most apparent during the beginning of lactation (Day 5) whereas the effects of AVPr1a antagonism did not become apparent until later (Day 15), indicating that other biological changes may moderate or alter these effects (Nephew & Bridges, 2008; Nephew et al., 2010).

There is evidence from multiple studies that AVP-ergic effects on aggression may be influenced by the serotonergic (5-HT) system and/or gonadal hormones. First, it appears that 5-HT may interfere with AVP-ergic induction of aggressive behavior in males. Delville, Mansour, and Ferris (1996a) found that intraperitoneal injections of fluoxetine (a selective 5-HT reuptake inhibitor) counteracted the effects of ventrolateral hypothalamic AVP administration on aggression; male golden hamsters receiving fluoxetine + AVP exhibited a higher latency to bite and fewer bites toward an intruder than those receiving only AVP (Delville et al., 1996a). In a followup study, investigators compared the effects of AVP, AVP + DPAT (a 5-HT 1a receptor agonist), and AVP + CGS (a 5-HT1b receptor agonist) in male golden hamsters order to determine whether serotonin's inhibition of AVP-ergic effects could be traced to a specific receptor subtype (Ferris, Stolberg, & Delville, 1999). Males receiving higher doses (10 or 100

μM) DPAT but not CGS exhibited reduced aggression (as indicated by latency to bite and number of bites), indicating a 5-HT1a receptor-specific effect.

In addition, several investigations found that androgen treatment in adolescent and adult male hamsters facilitated aggression and social dominance in conjunction with alterations in AVP-ergic neural systems (Delville, Mansour, & Ferris, 1996b; Ferris, Axelson, Martin, & Roberge, 1989; Grimes, Ricci, & Melloni, 2007; Harrison, Connor, Nowak, Nash, & Melloni, 2000). For example, Ferris and colleagues (1989) reported that testosterone treatment in pair-housed castrated males resulted in social dominance (indicated by flank marking, attacks, and bites) and a greater number of AVP-immunoreactive cell bodies in the nucleus circularis (of the anterior hypothalamus) compared to untreated castrated housemates (Ferris et al., 1989). Delville et al. (1996b) also found that ventrolateral hypothalamic injections of AVP reduced latency to aggressive behavior toward an intruder, but only in the presence of testosterone. Castrated animals lacking testosterone exhibited diminished ventrolateral hypothalamic-specific AVP1a receptor binding (Delville et al., 1996b). Perhaps not surprisingly, daily androgen treatment reduced 5-HT immunoreactive fibers and increased AVP immunoreactive fibers in the lateral anterior hypothalamus in a time-dependent manner, changes that coincided with a greater number of offensive attacks toward intruders (Grimes et al., 2007). Thus, it appears that AVP, 5-HT, and testosterone may interact in various ways to alter aggressive behavior. However, more information is needed to determine the nature of these interactions across species and identify with greater specificity the types of aggression affected by these interdependent systems.

Social dominance and aggression in humans.

As previously discussed in the context of social bonding, AVP may also affect humans' perceptions of, and reactions to, social stimuli. Some of these perceptual and behavioral affects

are likely to contribute to whether an individual is driven to respond in an aggressive manner. For instance, the effects of AVP have included enhancement of the recognition of angry faces (Guastella et al., 2010) and the alteration of patterns of facial responses to neutral stimuli to reflect responses to angry stimuli (Thompson et al., 2006; Thompson et al., 2004). In addition, cerebrospinal AVP levels have been positively associated with general aggression and aggression directed against others in individuals diagnosed with a personality disorder (Coccaro, Kavoussi, Hauger, Cooper, & Ferris, 1998). Clearly, however, much more research is needed to understand the role of AVP in aggression for both males and females across the lifespan. This topic will be discussed further in the introduction to Study 1.

Neuropeptide genes and behavior.

Thus far, candidate gene studies have primarily focused on the role of the receptor gene *AVPR1A* in socially-relevant behavioral phenotypes in humans. Following experimental evidence for the role of the AVPr1a receptor in various social phenotypes, including social recognition (Bielsky et al., 2005; Dantzer et al., 1987), pair bonding (Lim & Young, 2006), maternal behavior (Bosch & Neumann, 2008), and aggression (Cheng & Delville, 2009) in nonhuman animals, a large number of studies have examined the putative effects of variation within the *AVPR1A* gene on human social behavior. In humans, *AVPR1A* spans ~6.38 kilobases (kb) on Chromosome 12 and contains two coding exons situated on either side of a single 2.2 kb intron (Thibonnier et al., 1996). Several microsatellite repeat sequences have been located within the intron and 5' flanking regions (Thibonnier et al., 2000), two of which have been examined most frequently in the context of human social behavior: RS1, a $(GATA)_{14}$ tetranucleotide repeat is located 553 base pairs (bp) upstream, and RS3, a $(CT)_4$-TT-$(CT)_8$-$(GT)_{24}$ complex repeat 3625 bp upstream of the coding region (Thibonnier et al., 2000). In particular, the common 327 bp allele (analogous to the 334 bp in studies utilizing alternate DNA amplification methods) has been identified as a

variant of interest within RS3 based on significant findings for this allele across several studies (see Avinun et al., 2011). Findings for studies examining the effects of *AVPR1A* on human social behavior will be discussed in greater detail in the introductions to Studies 1 and 2.

The Missing Heritability Problem

The term "missing heritability" has been used to describe the disparity between the percentage of variance in many complex traits attributed to heritability (e.g., ~90% in autism; Freitag, 2007; ~50% in antisocial behaviorRhee & Waldman, 2002) and the variance that has actually been explained by specific genetic markers in molecular genetic studies. Studies examining individual markers within theoretically-relevant genes have generally shown only weak and/or inconsistent phenotypic associations that explain little of this heritability. In addition, recent genome-wide association studies, which examine variation indiscriminately across the entire genome, have not fared much better. An oft cited example illustrative of this phenomenon is that of height; although heritability has been estimated to account for ≥ 80% of the variability in height, conventional genome-wide association studies have implicated genetic loci that can only account for approximately 5% of its variability (Maher, 2008; Manolio et al., 2009). As illustrated by this example, failure to account for the vast majority of the estimated heritability in many traits and disorders of interest, including schizophrenia, autism, aggression, depression, and others, has stunned many, but potential explanations for this phenomenon include structural and rare genetic variation (The 1000 Genomes Project Consortium, 2012), epistatic effects (Zuk, Hechter, Sunyaev, & Lander, 2012), mosaicism, and epigenetics (Charney, 2012), among others.

Potential explanations aside, one might argue that given what we know about natural selection, it makes sense that we should expect common variation within the genome to have very small effects on human behavior (Plomin & Davis, 2009). Canalization theory (Waddington,

1959) posits that the physical properties of organisms maintain "a balance between flexibility and inflexibility" to external stressors over time, and this argument has been adapted by some geneticists to describe the process through which relative phenotypic stability of a trait may emerge in a population despite the presence of increasing genetic variability. Canalization may result when genotypes resulting in extreme phenotypic changes are selected against over time (Gibson & Wagner, 2000) producing a stable phenotype that shows restricted variability and is only weakly influenced by variation in individual genetic loci (Gibson & Wagner, 2000). Thus, if the common "risk" alleles exhibited strong, detrimental phenotypic effects (such as social deficits), these markers would likely have been selected against over time (Manolio et al., 2009; Plomin & Davis, 2009) and would thus appear only rarely in members of the population. This theory may account for why individual common variants within AVP-ergic genes have failed to yield large, replicable effect sizes for social phenotypes despite the theoretical support for these systems' influences in experimental research. We should thus not expect common, individual variants to yield large effect sizes in small to medium samples, but we need not discount the possibility that these variants may contribute small but detectable effects in large samples.

Another contributor to the problem of missing heritability may be the actual coverage of loci within these genes in the extant literature, which is generally very low (i.e., including only one or a few markers per study). Individual markers usually account for very little of the total variation within a particular gene, and a failure to adequately capture differences between individuals in gene expression due to polymorphisms across the gene has undoubtedly contributed to our inability to explain much variation within phenotypes of interest. For instance, in addition to the previously described microsatellites, there are over 100 SNPs within and immediately surrounding *AVPR1A* in humans of European ancestry. Although many of these SNPs are uncommon in this general population, at least 1/3 of the SNPs are present in greater

than 10% of the population (see Appendix A for information regarding the minor allele frequencies of *AVPR1A* and surrounding SNPs). Surprisingly, however, this variation has been largely unexplored in the extant literature. Thus, in order to better understand the roles of these genes in human social behavior, we must provide more comprehensive and consistent coverage of the variation across each gene.

More comprehensive coverage of candidate genes can be achieved through the exploitation of linkage disequilibrium (the nonrandom co-segregation of genomic loci in close chromosomal proximity) in the process of marker selection, genotyping, and analysis. By genotyping and then analyzing a carefully selected fraction of the markers that commonly vary within a gene of interest, we may be able to account for the majority of the variation within that gene without having to pay the literal and statistical costs of genotyping and analyzing every marker separately. Further, the concept of linkage disequilibrium (LD) can help us to understand why previous findings have appeared inconsistent and/or failed to replicate in the literature. For instance, a SNP that shows significant association with a phenotype of interest in one sample may merely be serving as a proxy for nearby loci responsible for the "true" effect. Consequently, if LD is not identical across samples, the examination of the "proxy" marker in a second sample may not yield the same pattern of association results. This observation may explain why findings for individual AVP-ergic loci have shown inconsistent associations with social phenotypes thus far. Indeed, following this same rationale, significant associations between a phenotype and two seemingly separate loci may actually emerge as the result of a single locus. It follows that we may be unaware of replications that have occurred in the extant literature because they have been disguised by associations with separate "proxy" loci.

Overall, it appears that providing maximal coverage of common, known variation within genes of interest with respect to LD may allow us to infer with greater confidence to what

extent these genes contribute to phenotypic variation in continuously-distributed population traits such as aggression or prosociality. In the following studies, we will utilize a novel gene-based method of examining the influence of *AVPR1A* on social phenotypes that takes into consideration variation across the gene while maximizing the power to detect significance in modest to moderate sample sizes.

AVPR1A and Aggression

Courtney A. Ficks and Irwin D. Waldman

Emory University

Abstract

The manipulation of central vasopressin (AVP) in nonhuman animals has been previously associated with changes in social aggression and dominance. Although multiple studies have examined the impact of intranasal AVP administration on social information processing and facial recognition, little is known about the impact of variation in the AVP-ergic system on human aggression. Because aggression appears to be an early risk factor for later negative outcomes, it may be particularly worthwhile to understand whether associations are present between AVP and aggressive behavior during childhood. The present investigation examined the effects of the AVPR1a receptor gene, *AVPR1A*, on childhood aggression using a novel gene-based, latent variable approach. Six hundred and twenty-one children ages 6-18 years recruited from several sources as part of an ongoing study of the etiology of common childhood psychiatric disorders were genotyped for 8 single nucleotide polymorphisms (SNPs) in *AVPR1A*. Parents reported on their children's aggressive behavior using Dodge & Coie's (1987) aggression scale. We examined various aspects of genotyping quality, including monozygotic twin discordance and Hardy-Weinberg equilibrium, before implementing a series of gene-based tests within a latent variable modeling framework. Aggression was modeled as a single latent factor indicated by the 12 aggression items using a confirmatory factor analytic approach. *AVPR1A* was also modeled as a single latent factor indicated by the genotyped SNPs (6, 7, or 8, depending on the model). The latent aggression factor was regressed on several covariates (age, sex, age^2, and ethnicity) as well as the latent *AVPR1A* factor. Results indicated significant associations for all models that included 7 or 8 SNPs and either significant or marginally significant associations for the 6 SNP models. We also tested an *AVPR1A* x sex interaction, which did not emerge as significant and thus provided no evidence that *AVPR1A*'s effects were sexually dimorphic. Overall, findings suggest that variation within the AVP-ergic system may play a small but

significant role in childhood aggression. Because this approach to modeling genetic associations allowed us to examine variation across *AVPR1A* without the elevation in Type I error resulting from conducting multiple statistical tests, future studies should consider this approach in examining genetic associations.

AVPR1A and Aggression

Aggression, or behavior that is "deliberately aimed at harming people and/or objects" (Kempes, Matthys, de Vries, & van Engeland, 2005) has been a widely studied phenomenon in the psychological literature, as aggressive behaviors are markedly elevated in clinical populations (American Psychiatric Association, 2000) and have the potential to put both the aggressor and his/her target(s) at risk. For children, aggressive behaviors are among the criteria for two of the most common behavioral disorders, oppositional-defiant disorder and conduct disorder, and these behaviors can put a child at risk for negative outcomes such as delinquency and/or emotional difficulties (Cleverley, Szatmari, Vaillancourt, Boyle, & Lipman, 2012) and even adverse health conditions later in life (Temcheff et al., 2011). Aggressive behavior has been shown to be moderately heritable (e.g., Burt & Neiderhiser, 2009; Rhee & Waldman, 2002), and there is evidence to suggest that serotonergic genes may play a small role in this heritability (see Ficks & Waldman, 2013 for meta-analysis). Nonetheless, the majority of the genetic variance in aggression has yet to be accounted for by known genetic markers.

The AVP-ergic system has been shown to play a role in aggression in nonhuman animals (Ferris, Meenan, Axelson, & Albers, 1986) and may be associated with aggressive behavior and/or threat perception in humans (Guastella, Kenyon, Alvares, Carson, & Hickie, 2010). AVP-ergic genes have been implicated in human social behavior, including autism, eating behavior, and substance use (Bachner-Melman et al., 2007; Kim et al., 2002; Maher et al., 2011), but there is little known about the effects of these genes on human aggression. These genes present promising targets for genetic association studies of aggression; as noted previously, the most commonly studied AVP-ergic gene, *AVPR1A*, codes for the AVPR1a receptor in humans and non-human animals, and experimental antagonism of the AVPR1a receptor has been shown to alter aggressive behavior in both male and female rodents (although the direction of these effects

may be sex and/or hormone-dependent; Bosch, Pfortsch, Beiderbeck, Landgraf, & Neumann, 2010; Cheng & Delville, 2009; Gutzler, Karom, Erwin, & Albers, 2010; Nephew & Bridges, 2008; Nephew, Byrnes, & Bridges, 2010). It is perhaps surprising then that so few studies have examined the potential effects of *AVPR1A* on aggressive or associated externalizing phenotypes in humans.

Two studies thus far have examined the role of *AVPR1A* in externalizing psychopathology. An association study of 1536 SNPs within 106 candidate genes revealed significant associations between 3 SNPs in *AVPR1A* (rs1587097, rs10784339, and rs11174811) and drug use disorder diagnoses in males and 1 SNP (rs1587097) and drug use disorder in females (Maher et al., 2011), indicating this gene may be involved in susceptibility to substance use and dependence. In a second study, Vogel et al. (2012) examined the AVR, RS1, and RS3 microsatellite repeats of *AVPR1A* within a sample of individuals with borderline personality disorder (BPD) and reported more impulsive aggression in those carrying "short" (210-216 bp) alleles of AVR and/or the "short" (193-218 bp) alleles of RS3. No significant effects were reported for the RS1 repeat. These studies provided some initial evidence that variation within *AVPR1A* may be associated with aggression and other forms of externalizing behavior, but clearly more research is needed to replicate and extend these findings.

The Current Study

The current study aimed to expand upon the existing literature in several ways. Primarily, we explored whether variation in the *AVPR1A* receptor gene was associated with variation in aggressive behavior in a sample of clinically-referred and non-referred children and their siblings. As noted previously, only two studies have examined the effects of this gene on externalizing behavior in adults, and to our knowledge, no studies have examined its effects in children. Based on the two existing studies as well as the aforementioned evidence that AVPR1a

receptor blockade can influence aggressive behavior in nonhuman animals, we hypothesized that variation in *AVPR1A* genotypes would be associated with variation in aggressive behavior in children.

In examining these associations, we also explored a novel method of testing genotype-phenotype associations that was designed to overcome several flaws present in traditional candidate gene studies. As noted in the general introduction, the "missing heritability" problem has plagued candidate gene research for more than a decade, and a major stumbling block in the search for causal variants for many complex traits has been researchers' inability to yield consistent, replicable genetic associations. Indeed, although there are too few studies available at this time to interpret the consistency of findings for the association between *AVPR1A* and aggression, related findings for the association between *AVPR1A* and autism (which will be discussed further in Study 2) have indeed been mixed and difficult to interpret. Some of this inconsistency in reported associations may be due to methodological differences between studies (e.g., examining "long" vs. "short" repeat lengths of RS3 instead of individual alleles or different SNPs within the gene, or using different phenotypic measurement criteria), but inconsistent findings may also result from 1) multiple testing or 2) differences in LD across study samples. Multiple testing increases the probability of Type I error when investigators do not adjust the criterion for rejecting the null hypothesis after conducting several independent statistical tests. Because candidate gene studies (including studies of *AVPR1A*) often test for associations with more than one microsatellite repeat or SNP, the probability of falsely rejecting the null hypothesis (i.e., committing a Type I error) is often inflated. Consequently, at least some of the significant "risk" alleles reported in these studies may have no real effect on the phenotype in the population of interest.

Further, as noted in the general introduction, LD may result in significant associations between a genetic marker and a phenotype when there is no "true" effect for that marker (i.e., the marker is only serving as a "proxy" for the nearby source of the effect). Because LD may differ to some extent between these samples, the different markers implicated in associations between studies may actually represent the single effect of an unmeasured source of variation in *AVPR1A*. Consequently, unless we are able to provide an alternative method of testing these associations that reduces the number of statistical tests conducted while taking into account differences in LD between markers across the gene, it will remain unclear whether variation in *AVPR1A* plays a significant role in aggression.

In an effort to examine the hypothesized associations while overcoming the common methodological flaws discussed above, we chose to conduct a gene-based test of the associations between *AVPR1A* and aggression by modeling *AVPR1A* as a latent factor indicated by genotyped SNPs that were selected to provide comprehensive coverage across the gene. This method utilized patterns of common variation across the gene (i.e., LD among the SNPs) to characterize individual differences in the latent gene factor while treating the gene itself as a unified whole, which allowed us to conduct a single, omnibus test of the hypothesized association.

Another aim of this study was to test whether the magnitude of associations between *AVPR1A* and aggression was sex-specific. Because findings of the effects of AVPR1a receptor blockade on behavior have been less consistent in females, perhaps as a result of hormonal modulation (Nephew & Bridges, 2008; Nephew et al., 2010), we expected that the overall associations between this gene and aggression would be greater in magnitude for males.

Method

Participants.

The current investigation genotyped a total of 621 children ages 6-18 years (mean age = 11.56, *SD* = 3.48 years) gathered from several sources. At-risk probands and their siblings were recruited from the Center for Learning and Attention Deficit Disorders and the Psychological Center at Emory University, both specialty clinics that provide assessment and treatment for families of children referred for learning, behavioral, and/or emotional problems. Diagnostic information collected by these clinics was not utilized in determining children's eligibility for inclusion in the current sample. In addition, a subset of twins from the Georgia Twin Registry, a representative sample of monozygotic and dizygotic twin pairs born in the state of Georgia between 1980 and 1991, were also recruited for participation in the current investigation. The ethnicity of participants in the current sample is as follows: 86.5% European, 7.3% African American, 2.3% Hispanic, and 3.9% of other or mixed ethnicity. Further information regarding the recruitment of these samples has been reported in previous publications (e.g., Rowe et al., 1998).

Participant data were collected during a 3-hour visit either in the laboratory (for the twins) or in participants' homes. During this time, the children completed an extensive battery of executive functioning assessments and provided saliva samples for DNA extraction while primary caregivers completed questionnaires assessing their children's behavior, temperament, and symptoms of common DSM-IV childhood psychiatric disorders.

Measures.

Aggression.

Aggression was measured via parent report on Dodge and Coie's (1987) 12-item aggression scale. Parents were asked to rate the extent to which various aggressive acts described their child's behavior, from 0 ("not at all") to 4 ("very well"). Previous investigations have demonstrated that more specific aggression dimensions ("reactive" and "proactive") may

be derived from this scale through principle-components factor analysis. For example, according to Dodge and Coie (1987), teacher reports of aggressive behavior across these 12 items yielded 3 items that loaded highly on the "reactive" aggression factor (e.g. "when teased, strikes back" ; factor loadings ranged from 0.70-0.86) and only moderately on "proactive" aggression (factor loadings ranging from 0.31-0.45), and conversely, 3 items that loaded highly on the "proactive" aggression factor (e.g. "gets others to gang up on a peer"; factor loadings ranged from 0.64-0.84) and only moderately on "reactive" aggression (factor loadings ranged from 0.33-0.61). Nonetheless, some have described this classification of aggression as "reactive" or "proactive" as an arbitrary oversimplification of a more complex phenotype (Bushman & Anderson, 2001), and considerable overlap has indeed been found between these dimensions (r = .76; Dodge & Coie, 1987). Indeed, we found a strong correlation between reactive and proactive aggression scale scores in our own data (r = 0.69, p < 0.001), and a reliability analysis of the the full 12-item Dodge and Coie (1987) scale demonstrated high internal consistency (Cronbach's α = 0.925) and split-half reliability (Spearman-Brown r = 0.92) among all items. For these reasons, in the current investigation we chose to analyze aggression as a unitary construct.

Genotyping.

Participant DNA was collected from Buccal cells using a 30-mL solution of 4% sucrose, which participants were asked to rinse in their mouths for 1 minute. Following collection, samples were labeled and immediately refrigerated and transported to the laboratory for preservation. Samples were later transferred to the Ressler laboratory at Emory University for genotyping. DNA was quantified by gel electrophoresis using Quantity One (BioRad, Hercules, CA). DNA concentrations were normalized to 10 ng/µl and were not used if they fell below 5 ng/µl. DNA was plated at 10 or 20 ng for Taqman (for rs11174803) or Sequenom (for rs962862, rs1587097, rs2738250, rs4763062, rs11174808, rs11174820, and rs7307997) for genotyping.

Taqman SNP Genotyping Assays and Taqman Genotyping Master Mix (Applied Biosystems Inc., Foster City, CA) and the ViiA7 Real Time PCR System were used to generate Taqman genotypes, and the iPLEX chemistries and the MassARRAY system (Sequenom, Inc., San Diego, CA) were used to generate Sequenome genotypes. Samples were duplicated within-plate for quality control to assess assay integrity.

Imputation.

The imputation of missing genotypes for *AVPR1A* was completed using the BEAGLE Genetic Analysis Software Package (Browning & Browning, 2009). BEAGLE uses Haplotype Hidden Markov Models (HHMs) to infer genotypic probabilities for missing SNP data in a sample of unphased individuals (i.e., those for whom information regarding the cosegregation of alleles on the same chromosome is missing) given observed haplotypes in a known dataset of phased individuals (i.e., a reference panel of individuals for whom information regarding the cosegregation of alleles on the same chromosome is known; Browning & Browning, 2009). The HHMs are built using an iterative algorithm that incorporates sampling observed genotypes and model-building (Browning & Browning, 2009). The reference panel used for imputation in the current investigation included genomic sequence data collected by the 1000 Genomes Project, a freely available standard resource on common variation across the genome that has collected samples from over 1000 individuals across 14 populations (The 1000 Genomes Project Consortium, 2012). The most probable genotype for an individual can be inferred from that individual's allelic *dosage* score provided by BEAGLE, which is calculated from the genotypic probabilities for a SNP with 2 common alleles (A and B) as follows:

$$Dosage = 0 \times P(AA) + 1 \times P(AB) + 2 \times P(BB)$$

All imputed dosage scores were converted to genotype scores in order to ensure that the imputed (missing) data and raw (known) data were handled by analytic software in a

consistent manner. Individuals whose imputed allelic dosage scores were less than .5 were coded as 0 (homozygous for the minor allele), individuals whose dosage scores ranged from .50 — 1.49 were coded as 1 (heterozygous), and individuals whose dosage scores were greater than or equal to 1.5 were coded as 2 (homozygous for the major allele). Imputation accuracy was gauged using the allelic R^2 provided by BEAGLE, which is calculated as the squared correlation between the number of minor alleles for the imputed genotype and the number of minor alleles for the true genotype (Browning & Browning, 2009). When unknown, true genotypes can be estimated from the posterior probabilities of the imputed genotypes (Browning & Browning, 2009).

Analyses

Assessing genotyping quality.

We examined the genotyping quality for each gene of interest in the following ways. It is important to note that although we conducted these analyses on the full sample of genotyped individuals (which included both children and their parents), only the children's data was utilized in the modeling of the associations between *AVPR1A* and aggression.

Call rate.

We first calculated the call rate for each SNP within a gene as the percentage of individuals who were successfully genotyped for that SNP divided by the total number of individuals for whom genotyping was attempted.

$$SNP\ Call\ Rate = \frac{Number\ of\ individuals\ successfully\ genotyped}{Number\ of\ individuals\ successfully + unsuccessfully\ genotyped}$$

We then calculated the call rate for each participant in the sample as the total number of SNPs successfully genotyped for that participant divided by the total number of SNPs in the gene (see equation below). In order to screen out DNA samples with poor genotyping yield, we identified participants with individual call rates less than 65% for removal in subsequent

analyses. Because genotyping of 8 SNPs was attempted for *AVPR1A* and successful genotyping of 5/8 SNPs for *AVPR1A* yielded a call rate that approached this cutoff (62.5%), we adopted this slightly lower cutoff for the individual call rate of *AVPR1A*.

$$Individual\ Call\ Rate = \frac{Number\ of\ SNPs\ successfully\ genotyped\ within\ each\ gene}{Number\ of\ SNPs\ successfully + unsuccessfully\ genotyped}$$

Monozygotic twin discordance rate.

Because monozygotic (MZ) twins are genetically identical, the genotypes of MZ twins should be identical for all SNPs within a gene. MZ discordance rates were calculated for each SNP as the number of MZ twin pairs exhibiting differing genotypes divided by the total number of MZ twin pairs successfully genotyped for that SNP.

$$\%\ MZ\ Discordance = \frac{Number\ of\ discordant\ MZ\ twin\ pairs}{Number\ of\ successfully\ genotyped\ MZ\ twin\ pairs}\ x\ 100$$

Hardy-Weinberg equilibrium.

Hardy-Weinberg Equilibrium (HWE; Hardy, 1908; Hosking et al., 2004) describes the expected number of heterozygotes in a population given the proportion of homozygotes of the major and minor alleles for a given SNP. HWE is a commonly examined index of potential genotyping error, and a Pearson chi-squared test with 1 degree of freedom (df) may be used to test the significance of deviation from HWE. We tested deviation from HWE for each SNP in the current investigation using Pedstats software (Wigginton & Abecasis, 2005), which provides basic summary statistics for datasets containing genetic pedigree information. Pedstats performs an *exact* test of HWE by computing a probability distribution of expected genotypes for each SNP conditional on the minor allele frequency and using that distribution (rather than the traditional 1-df chi squared distribution) to determine the significance of deviation from HWE. Exact HWE tests were conducted 1) in the full sample, 2) in a randomly selected sample of unrelated individuals from the full sample, and 3) in founders only (the parent generation of the

pedigrees). In order to infer significant deviation from HWE, we selected an alpha level corrected for the number of significance tests conducted per gene (i.e., the number of SNPs per gene).

$$\alpha = \frac{0.05}{Number\ of\ SNPs\ tested\ per\ gene}$$

It is important to note that in clinical samples that include "affected" individuals (or samples selected for the trait of interest), deviation from HWE may actually be indicative of association between the genotyped SNP and the selected trait rather than genotyping error (Nielsen, Ehm, & Weir, 1998). Further, the inclusion of non-independent observations (i.e., families with correlated genotypes) in the test for HWE may bias findings. Consequently, comparisons of deviation from HWE among "all individuals" with deviation in the "unrelated" and "founders only" groups can provide information regarding whether deviations in HWE may be driven by the preponderance of affected individuals in the sample or the presence of non-independent observations.

Minor allele frequency.

The frequency of the minor (less common) allele of a SNP in the sample was defined as the number of minor alleles in the sample out of the total number of alleles. The minor allele frequency (MAF) found for each SNP in the current study's sample was compared with the MAF previously found in the International HapMap Project's (Thorisson, Smith, Krishnan, & Stein, 2005) European (CEU) and African (YRI) samples.

$$MAF = \frac{Number\ of\ minor\ alleles\ in\ the\ sample}{Total\ number\ of\ alleles}$$

Or

$$MAF = \frac{(2\ x\ minor\ allele\ homozygotes) + heterozygotes}{2\ x\ (minor\ allele\ homozygotes + heterozygotes +\ major\ allele\ homozygotes)}$$

Modeling genotype-phenotype associations.

We used Mplus statistical software version 7.0 (Muthén & Muthén, 1998-2012) to conduct gene-based tests of the hypothesized associations between *AVPR1A* and aggression. The goal of these gene-based tests was to determine to what extent common variation across the *AVPR1A* gene contributes to variation in aggressive behavior in children. First, we used confirmatory factor analysis (CFA) with a robust weighted least squares estimator (i.e., WLSMV) to model aggression as a normally-distributed latent factor indicated by the ordinal Dodge and Coie (1987) scale items (see Appendix B). The fit of this measurement model and the significance of factor loadings were assessed prior to the factor's inclusion in the overall structural model. Next, the aggression phenotype factor was regressed on a series of covariates (age, age^2, sex, age X sex, age^2 X sex, proportion European ancestry, proportion African ancestry, and proportion Hispanic ancestry) in order to reduce variability in aggression due to these participant characteristics. In the presence of strong multicollinearity, we reduced the covariates in this model to a more manageable subset (age, age^2, sex, proportion European ancestry). Next, we modeled *AVPR1A* as a normally distributed latent genetic factor with the eight genotyped SNPs as categorical factor indicators. The fit of the measurement model for *AVPR1A* and significance of individual factor loadings were also assessed prior to the latent gene factor's inclusion in the overall structural model.

Goodness of fit was judged using multiple indices provided by Mplus statistical software:

1) The Akaike information criterion (AIC) and the Bayesian information criterion (BIC) are both commonly used indicators of comparative model fit and parsimony that take into account factors such as predictive accuracy, sample size, and the number of parameters in the model, and lower values for these indices are interpreted as more favorable (Akaike, 1974; Schwarz, 1978). The calculation of these indices is as follows,

$$AIC = 2k - 2LL$$

$$BIC = -2LL + k * \ln(N)$$

where *k*= the number of estimated parameters in the tested model, *2LL* = 2 x the log likelihood of the tested model, and N = sample size.

2) Several fit indices that are based on the noncentrality parameter *d*, which takes into account both deviation from the saturated model (i.e., a model that allows for associations between all observed variables) and sample size, were also utilized,

$$d = \chi^2 - df$$

where χ^2 is the difference in *-2LL* between the tested model and the saturated model and *df* indicates the number of variances of and covariances among the observed variables minus the number of freely estimated parameters in the tested model.

a. The root mean square error of approximation (RMSEA) is a measure of absolute fit (Loehlin, 2003), with RMSEA ≤ .08 commonly interpreted as indicating adequate fit and values closer to 0 indicating increasingly good fit (Browne & Cudeck, 1993),

$$RMSEA = \sqrt{\frac{d}{(N-1) \times df}}$$

where *df* indicates the number of variances of and covariances among the observed variables minus the number of freely estimated parameters in the tested model, and N = sample size. The comparative fit index (CFI) and the Tucker-Lewis index (TLI) provide standardized indices of model fit while taking into account model parsimony and are akin to the R^2 in multiple regression; values greater than 0.950 may be interpreted as reasonably good fit (Hu & Bentler, 1999),

$$CFI = 1 - \frac{d_{tested\ model}}{d_{baseline\ model}}$$

$$TLI = 1 - \frac{d_{tested\ model}}{d_{baseline\ model}} \times \frac{df_{baseline\ model}}{df_{tested\ model}}$$

where the baseline model indicated is a null model in which all associations between all observed variables are assumed to be zero and *df* indicates the number of variances of and covariances among the observed variables minus the number of freely estimated parameters in the tested model.

3) The standardized root mean square residual (SRMR) is an index of the difference between the predicted and observed variances of and covariances among the variables, taking into account the number of parameters in the model. SRMR < .08 is an accepted cutoff for good fit (Hu & Bentler, 1999). Please refer to the Mplus technical manual (Muthén & Muthén, 1998-2012) for the complex formula for SRMR.

Finally, the latent aggression factor was regressed on both the aforementioned covariates and the latent *AVPR1A* factor in order to model the hypothesized association between *AVPR1A* and the phenotype of interest (i.e., aggression). Refer to Figures 1-5 below for a visualization of each model. A *z*-test was used to assess the significance of the regression coefficient of the latent aggression factor on the latent *AVPR1A* factor with respect to its standard error. In addition, the R^2 contributed by the *AVPR1A* factor (or by the 8 *AVPR1A* SNPs) to the latent aggression factor was computed as the difference in R^2 for the full structural model and that of the model containing the latent aggression factor and its covariates, but not *AVPR1A*.

Sex differences in *AVPR1A*-aggression associations.

We tested whether sex differences were present in the magnitude of associations between *AVPR1A* and aggression by adding an interaction term (*AVPR1A* X sex) into the final, best-fitting structural model. The significance of the interaction term was determined using a z-test of its regression coefficient with respect to its standard error.

Results

Genotyping Quality

We repeated quality control analyses on the full sample as well as several subsamples after removing cases for the reasons described below. See Table 1 for information regarding sample sizes and basic demographics across the subsamples. Results for the genotyping quality control analyses in each sample/subsample are reported in Tables 2-6.

1) Full sample. We first performed quality control analyses on the full sample of genotyped individuals (including both children and parents). See Table 2 for results.
2) High-quality subsample. In order to screen out those with potentially low-quality DNA samples, we removed individuals for whom the percentage of successfully genotyped SNPs for *AVPR1A* fell below the previously described cutoff (62.5%). This resulted in the removal of approximately 181 (~16%) genotyped individuals from the full sample. See Table 3 for results.
3) High-quality subsample, European ancestry. Because different minor allele frequencies (MAFs) were observed between the European and African samples from the International Hapmap Project for nearly all SNPs examined in the current study, we selected a subsample of individuals with less than 50% African Ancestry in order to examine the potential influence of population stratification on our analysis of genotyping quality. This resulted in the removal of an additional 52 (~5%) individuals from the high-quality subsample. See Table 4 for results.
4) Imputed full sample. Reliable imputation of missing genotypes for the SNPs of interest may increase the number of available observations per SNP and consequently our statistical power to detect associations between *AVPR1A* and aggression. We utilized the program Beagle (Browning & Browning, 2009) to estimate the missing genotype

data for the full sample using each individual's available genotypic information in conjunction with known patterns of LD across *AVPR1A* from the 1000 Genomes database (The 1000 Genomes Project Consortium, 2012). We then performed quality control analyses on the imputed genotypes in order to gauge the quality of these data. See Table 5 for results.

5) Imputed high-quality subsample. Because imputed data for individuals with low genotyping yields (i.e., fewer than 5 of 8 SNPs successfully genotyped) may be less reliable than imputed data for individuals with higher genotyping yields, we mirrored analysis 2) above with the dataset containing imputed genotypes by screening out those individuals whose genotyping had initially been judged as "poor" prior to imputation. Thus, we utilized Beagle software to estimate missing genotypes for individuals missing 3 or fewer genotyped SNPs (~84% of the sample), and the remaining individuals were removed from the analysis. See Table 6 for results.

Call rates and reliability of imputation.

The call rates for each SNP in the full sample and each subsequent subsample are reported in Tables 2-6. In the full sample, the average call rate per SNP was 83.49%. In the high-quality subsample, the average call rate per SNP improved to 96.73%. The inclusion of only individuals of European ancestry in the high-quality subsample did not produce much difference in the call rate per SNP (96.69%). For the imputed data, reliability of imputed SNP data as indicated by R^2 (the squared correlation coefficient for the association between the estimated and true genotypes, provided by Beagle software following imputation) was fair to good, ranging from 0.74-0.97 for imputed SNPs in the full sample. In the high-quality subsample, the R^2 for imputed SNPs in the subsample was very good (0.93-0.99).

MZ twin discordance.

The average rate of MZ discordance was fairly low in the full sample (2.43%), and it was further reduced in the subsamples (2.22% in both). Although the MZ discordance rate for most SNPs ranged from 0-3%, one SNP (rs1587097) exhibited a consistently higher MZ discordance rate (~6%). In the imputed full sample, the average MZ discordance rate increased to 7.85%. In the imputed high-quality subsample, the average MZ discordance rate was improved (3.93%), although it was still slightly worse than the MZ discordance rate observed in the raw data.

Hardy-Weinberg equilibrium.

In examining all probands and their relatives (i.e., "all individuals") in the full sample, many SNPs (5/8) showed significant deviation from HWE (i.e., $p < .05 / 8 = .00625$), indicating the proportion of heterozygous individuals per SNP differed from what would be expected given the minor allele frequency. However, when this test was performed exclusively on unrelated individuals or on founders within the full sample, only 2 of these SNPs continued to show significant deviation from HWE (only 1 of which was significant in both the unrelated and founders samples), suggesting that LD and/or the presence of non-independent observations (i.e., participant relatedness) contributed to deviations from HWE for multiple SNPs among all individuals.

In the high-quality subsample, 4/8 SNPs showed significant deviations from HWE among all individuals, whereas only 1 of 8 SNPs showed significant deviation from HWE among unrelated individuals and founders (the deviating SNP differed between these two groups).

Finally, in the high-quality subsample of European ancestry, 4 SNPs showed significant deviation from HWE among all individuals whereas only 1 SNP showed significant deviation among unrelated individuals and no SNPs showed significant deviation among founders. Again, this suggested that LD and/or non-independent observations may have contributed to deviations in HWE observed for multiple SNPs.

For the imputed data, the number and pattern of HWE deviation for all individuals, unrelated individuals, and founders within the full sample was similar to that observed in the sample prior to imputation, although there were a few differences in which SNPs exhibited deviation. In the high-quality imputed subsample, 4 SNPs deviated from HWE among all individuals but no SNPs deviated from HWE among unrelated individuals or founders, suggesting that LD and/or non-independent observations again resulted in the aforementioned deviations among all individuals.

Minor allele frequency (MAF).

The MAFs for SNPs in the current study were similar to those previously reported for individuals of European ancestry by the International HapMap Project (see Tables 2-6). The average absolute difference in frequency between the full sample in the current study and the HapMap sample across SNPs was 2.8%. For the high-quality subsample and the high-quality European ancestry subsample, respectively, the average absolute deviations in MAF from that reported in the HapMap sample were similar (average absolute difference ~2.9-3.1%). For the imputed data, the average absolute differences in MAF for the full sample and the high-quality subsample were slightly higher (~3.2-3.4%).

Modeling *AVPR1A* and Aggression

Overall, findings from the quality control analyses indicated that 1) removing individuals from the sample with poor genotyping success improved genotyping quality but reduced the sample size by ~16% overall, and 2) using imputation to replace missing data increased the number of genotyped SNPs available for analysis but had a slight negative impact on genotyping quality (most noticeable in the elevated MZ discordance rates). Based on these findings, we chose to fit the proposed models of association between *AVPR1A* and aggression in both the raw data and the imputed data. Further, for each data type, we tested whether findings differed

between the full sample and the high-quality subsample. Below is an outline of the samples used in association testing:

1) Raw data
    a. Full sample
    b. High-quality subsample
2) Imputed data
    a. Full sample
    b. High-quality subsample

Testing the models under these two sets of mirrored conditions allowed us to determine the sensitivity of parameter estimates to the tradeoffs in genotyping quality and statistical power. Standard practices regarding the modeling of latent genetic effects have yet to be established, so by providing this information we hoped to inform methodological decision-making in future investigations with similar aims.

Fit statistics and standardized regression coefficients for these models of the association between *AVPR1A* and aggression are shown in Table 7. Findings for the models separated by sample type are discussed below.

Raw data.

Full sample.

In the measurement model of aggression, all 12 Dodge and Coie (1987) items loaded significantly on the latent aggression factor, and fit statistics indicated that the model fit the data well. Nonetheless, there was strong multicollinearity among the covariates, and as a result none of the covariates were significantly associated with the latent aggression factor. Reducing the number of covariates in the model (see Methods) to age, age^2, sex, and proportion of European ancestry improved model parsimony and reduced multicollinearity among predictors

without significantly reducing model fit, and all covariates in this subset emerged as significant predictors of the latent aggression factor in this reduced model except for the proportion of European ancestry. We included this covariate in subsequent models in order to ensure the absence of population stratification effects on associations between *AVPR1A* and aggression.

In the measurement model of *AVPR1A*, all SNPs loaded significantly on the latent *AVPR1A* genetic factor. Even so, the fit statistics for this model, which describe to what extent the latent factor "holds together" given the factor indicators (in this case, the genotyped SNPs), indicated fairly poor model fit. Poor model fit may occur when some of the factor indicators are more strongly correlated than others and when the data may be better represented by more than one factor. Indeed, within this model, some of the correlations between SNPs due to LD approached 1, indicating near perfect association, while other correlations were less than 0.7. As a result, Mplus statistical software produced a warning indicating high correlations between some SNPs (r = -.999) and problems with model convergence. We tested the impact of removing individual SNPs from the model on model fit by dropping the 2 SNPs that exhibited unusually high correlations with other SNPs within the model in two separate steps (rs1174808 followed by rs1587097). The removal of each of these SNPs resulted in observable improvements in model fit (see Table 7). Because our goal was to model all known variation across the gene in a single gene-based test of significance, however, it was unclear whether removing one or more SNPs from the model for the purpose of creating a more "unified" latent factor was a desirable alternative. Indeed, the removal of known sources of variation within the gene may result in a less comprehensive and accurate characterization of differences in the proteins (i.e. the AVPR1a receptors) expressed by variants of this gene. Thus, for the subsequent structural models, we chose to include all 8 SNPs in the initial test of association and then drop each of the 2 highly

correlated SNPs in subsequent nested models to observe the impact on model fit and on the associations of interest.

The full structural model, which included the latent aggression factor with 12 indicators, the reduced set of covariates, and the latent *AVPR1A* genetic factor with 8 SNP indicators, fit the data well according to model fit statistics. The coefficient for the regression of aggression on *AVPR1A* revealed a significant association, B = -.094, SE = .042, z = -2.25, p = .024, R^2 = .011. Removing the first highly correlated SNP, rs1174808, as an indicator of *AVPR1A* improved model fit and increased both the regression coefficient and its standard error slightly while only slightly decreasing the significance of the association, B = -.113, SE = .058, z = -1.96, p = .050, R^2 = .016. Removing the second SNP, rs1587097, as an indicator of *AVPR1A* had a slight negative impact on the regression coefficient, resulting in a marginally significant association between *AVPR1A* and aggression, B = -.106, SE = .059, z = -1.78, p = .076 R^2 = .014.

Finally, we tested whether associations between *AVPR1A* and aggression differed for males and females by including an *AVPR1A* X sex interaction term in the final model. The interaction term did not emerge as significant (z = -1.24, p = .214), providing no evidence for sex-specific effects.

High-quality subsample.

As noted previously, removing poorly genotyped individuals from the full sample of children and their families reduced the sample size by 181 (~16% of the sample). Because only child data were utilized for the modeled genetic associations, removing poorly genotyped individuals from the sample resulted in a loss of data for 86 participants for these analyses, or ~15% of the sample.

As in the full sample, for the measurement model of aggression all 12 items loaded on the latent aggression factor in the high-quality subsample and exhibited excellent fit. For the

measurement model of *AVPR1A*, fit statistics for the model that included all 8 SNPs were slightly better than previously seen in the full sample, and again, dropping the two aforementioned SNPs from this model improved the fit of the model.

The pattern of findings in the structural models was also similar to that seen in the full sample. Fit statistics indicated the initial model fit the data well, although dropping the 2 highly correlated SNPs from the model resulted in small improvements in model fit and parsimony. The regression coefficients for the models using the high-quality subsample were slightly higher than they were for the full sample, but the reduction in *N* resulted in slightly higher standard errors for these coefficients, resulting in less significant or marginally-significant associations for all three models (B = -.112, SE = .059, z = -1.92, p = 0.055, R^2 = .015 for 8 SNPs, B = -.113, SE = .058, z = -1.94, p = 0.052, R^2 = .015 for 7 SNPs, B = -.103, SE = .060, z = -1.73, p = 0.084, R^2 = .013 for 6 SNPs).

Once again, the *AVPR1A* X sex interaction term did not emerge as significant, thus failing to provide evidence that the associations between *AVPR1A* and aggression differed by sex, z = -1.38, p = 0.168.

Imputed sample.

Many of the individuals included in the full sample had data that were missing for one or more SNPs. Out of 4,984 SNPs for which genotyping was attempted (623 individuals X 8 SNPs), data for 757 SNPs were missing (~15% of all genotyped SNPs). Following imputation, although our overall sample *N* did not change, we gained a substantial amount of data that was previously unavailable for testing the genetic associations of interest.

Fit statistics for the models that utilized the imputed genotype data were comparable but slightly worse than the fit statistics for the models that utilized the raw genotype data, likely due (at least in part) to the increased number of observations for each SNP variable. In the

measurement model for *AVPR1A*, all SNPs loaded significantly on the latent genetic factor. In the structural model containing both the *AVPR1A* and aggression factors, fit statistics indicated excellent fit to the data. Interestingly, the regression coefficients for the models that utilized imputed data were somewhat higher than those seen in previous models, although the standard errors remained the same. This resulted in significant associations between *AVPR1A* and aggression for all models (even after removing the 2 highly correlated SNPs from the model), B = -.122, SE = .048, z = -2.58, p = .010, R^2 = .018 for 8 SNPs, B = -.130, SE = .055, z = -2.37, p = .018, R^2 = .021 for 7 SNPs, B = -.122, SE = .056, z = -2.19, p = .029, R^2 = .018 for 6 SNPs.

Again, the *AVPR1A* X sex interaction term introduced in the final model did not emerge as significant, providing no evidence for differences in these associations between males and females, z = -1.37, p = .172.

Imputed high-quality subsample.

Fit statistics for the models that utilized imputed data for the high-quality subsample were somewhat more favorable than the fit statistics for any of the previous conditions. For the measurement model of *AVPR1A*, 7 of the 8 SNPs (the exception being rs1587097) loaded significantly on the latent genetic factor. When the two highly correlated SNPs were subsequently dropped from the measurement model, 6/7 and 5/6 SNPs, respectively, loaded significantly on the latent genetic factor. In the structural model, regression coefficients and standard errors for the imputed high-quality sample were similar to those seen in the high-quality sample prior to imputation. *AVPR1A* emerged as a significant predictor of aggression in the two models utilizing 8 and 7 SNPs (B = -.119, SE = .059, z = -2.03, p = .043, R^2 = .017 and B = -.115, SE = .059, z = -1.95, p = .051, R^2 = .016, respectively), but was only marginally associated with aggression in the model containing 6 SNPs (B = -.099, SE = .060, z = -1.64, p = .102, R^2 = .012).

Once again, the *AVPR1A* X sex interaction term did not emerge as significant $z = -1.32$, $p = .187$.

Testing individual SNPs.

Although our primary goal in the current investigation was to comprehensively examine the combined impact of genetic variation across *AVPR1A* on aggression in children, we sought to contrast these gene-based analyses with a set of additional SNP-based analyses that utilized a more traditional approach to examining genotype-phenotype associations. For these complementary SNP-based analyses, instead of regressing the latent aggression factor on a latent *AVPR1A* factor indicated by the genotyped SNPs (as in the aforementioned structural models), we regressed the latent aggression factor on the 8 genotyped SNPs directly. See Figure 6 for an illustration of this model. This method allowed us to determine whether any of the individual SNPs in *AVPR1A* contributed *unique* variance in aggression over and above that contributed by common variation (i.e., reflecting LD) across the gene, which was tested in the above models.

In order to treat the genotyped *AVPR1A* SNPs as predictors within the model, we recoded the SNPs as binary categorical variables given the very low frequency of genotypes comprising two copies of the minor allele. Heterozygous genotypes or genotypes that were homozygous for the minor allele were coded as 1 for each SNP, whereas individuals whose genotypes were homozygous for the major allele were coded as 2. Unlike the gene-based tests, the SNP-based tests required 8 separate significance tests within each model (i.e., one test for the regression of aggression on each SNP). We applied the Bonferonni correction to our criterion alpha ($\alpha = .05$) in order to compensate for this multiple testing, resulting in a SNP-based critical $p = .00625$.

See Table 8 for the results of these SNP-based tests. Because SNPs were treated as predictor variables in these models (as opposed to the aforementioned models, which treated them as dependent factor indicators)—and models excluded individuals who did not have information available on all predictor variables—fit statistics and parameter estimates from the SNP-based tests were identical for the full sample and the high-quality subsample, so we reported them jointly. Using the Bonferonni-corrected criterion, only one measured SNP, rs11174803, exhibited a unique association with latent aggression scores in the raw data samples, z = -2.96, p = .003. No SNPs emerged as significant predictors of latent aggression scores in either of the imputed data samples.

Discussion

The primary goal of this study was to examine associations between *AVPR1A* and childhood aggression using a novel gene-based approach. Prior to examining these associations, we conducted an in-depth assessment of the quality of genotyping for our sample. In the full sample, we found that genotyping quality as indicated by MZ discordance rate and minor allele frequency for each of the SNPs was fairly good, but call rates for most of the SNPs were less than 90% and there was some significant deviation from Hardy-Weinberg equilibrium. Following these observations, we removed individuals from the sample whose low genotyping success rates may have been indicative of poor quality DNA samples. The removal of these individuals from the high-quality subsample improved nearly all indices of genotyping quality but reduced our sample size considerably (~16%), thus reducing our power to detect associations in subsequent analyses. In an attempt to maximize both genotyping quality and statistical power, we then tested how the imputation of missing values for both the full sample and the high-quality subsample impacted indices of genotyping quality. Although this imputation increased the number of available observations per SNP, it also resulted in elevated MZ discordance rates

for multiple SNPs, bringing the quality of the imputed data into question. Overall, it appeared that each deviation from the original raw data resulted in either 1) improved genotyping quality and reduced statistical power, or 2) reduced genotyping quality and improved statistical power. Fortunately, we observed in subsequent analyses that our findings were robust to these tradeoffs in genotyping quality and statistical power, although these characteristics did impact somewhat the significance of associations within the 6 SNP models. We recommend that future genetic association studies continue to assess genotyping quality using more than one index, as the use of multiple indices allowed us to observe changes in quality within our own data following subsample selection and imputation that may have otherwise gone unnoticed.

Based on previous findings from experimental research on the effects of AVP-ergic manipulation in humans and non-human animals as well as two genetic association studies that examined the effects of *AVPR1A* on externalizing behavior, we hypothesized that common variation in *AVPR1A* would be associated with variation in human aggression. Our findings supported this hypothesis, as the latent genetic factor for *AVPR1A* was significantly associated with the latent aggression factor in nearly all of the models that we tested (and was marginally associated in the remaining models), and this association was robust to changes in sample size and the number of observations available for each measured SNP. The amount of variance in aggression explained by the latent *AVPR1A* factor ranged from 1.1-2.1%, depending on the sample type and the number of SNPs included in the model. Although the estimates provided indicate that the gene plays only a small role in the etiology of aggression overall, it is important to note that the magnitude of "risk" conferred by variation in this gene is relatively large compared to that which is typically found for individual loci in candidate gene studies or genome-wide association studies (GWAS) of complex traits (Manolio et al., 2009). Individual SNPs identified in GWAS most often exhibit odds ratios less than 1.45, thus contributing 1% or

less of the variance in the phenotype(s) of interest (Manolio, 2010). These findings support the notion that more comprehensive coverage of SNPs within genes may help to explain some of the "missing heritability" in complex phenotypes.

Further inspection of the magnitude of associations within these models revealed that the nature of the reported associations was also largely consistent across models. The models that utilized 7 or 8 SNP indicators for the latent *AVPR1A* factor always yielded regression coefficients and standard errors that were similar in magnitude and produced significant associations between *AVPR1A* and aggression. The models that utilized 6 SNP indicators for the latent genetic factor, in contrast, yielded somewhat lower regression coefficients but similar standard errors, producing a pattern of associations between *AVPR1A* and aggression that bordered between significant and marginally-significant. Because the only difference between the 6 SNP models and their predecessors was the removal of rs1587097 as an indicator of the latent genetic factor, these observations may indicate that rs1587097 contributes to variation in *AVPR1A* that is associated with incremental variance explained in aggressive behavior. In support of this, as noted in the introduction to this study, variation in rs1587097 has been previously associated with drug use disorders in both males and females (Maher et al., 2011) and may consequently represent a region of the gene that contributes to externalizing behavior. Nonetheless, more research is needed to replicate these associations and help us to better understand their nature. Overall, these findings also demonstrate the importance of vigilance in selecting markers for inclusion in latent gene-based models, as marker selection can impact the pattern of observed effects.

We also inspected the magnitude and direction of factor loadings and associations across models, which further reaffirmed the consistency of our findings. For the latent aggression factor, mother- and father-reported aggression items always showed positive,

significant factor loadings. For the latent *AVPR1A* factor, 5 of 8 SNPs consistently loaded significantly on this factor in the same direction across models (with rs11174803, rs1587097, rs2738250, and rs7307997 always displaying positive factor loadings and rs962862 always displaying a negative factor loading). Two of the remaining 3 SNPs (rs1174808 and rs1174820) displayed significant negative and positive factor loadings, respectively, but only for the models that utilized the full sample (raw or imputed). The remaining SNP (rs4763062) only loaded significantly (in the negative direction) on the latent gene factor in the model that utilized the full sample with imputed data. Although we have listed the significance and direction of these factor loadings here in order to demonstrate consistency in the measured constructs of interest and the nature of their relations across models, it is important to note that we should not use this information to make inferences regarding the magnitude and/or direction of associations between individual SNPs in *AVPR1A* and aggression. Because only a single gene-based test was conducted, we can only infer that at least *one* source of variation within the gene was significantly associated with increased aggression. The remaining sources of variation within the gene, although related to the source of significant association through LD, may have had no real effect on the phenotype of interest. Consequently, although gene-based tests such as this can allow us to determine with greater confidence whether a candidate gene is associated with a phenotype of interest, we should be cautious in drawing conclusions about whether specific alleles within the gene serve as “risk” or “protective” factors for that phenotype.

Given the novelty of our gene-based tests of association using a latent genetic factor, we conducted complementary analyses to contrast our results with those obtained using a more traditional SNP-based approach within the structural models. The SNP-based approach differed from the gene-based approach in that it yielded separate significance tests for each SNP within the model. Because all SNPs were examined within the same model, covariation among the

SNPs (due to LD) was taken into account, and consequently any associations between a SNP and aggression were indicative of unique effects of that SNP. In the SNP-based tests, only one SNP (rs11174803) demonstrated unique effects (at $p < .05$) for both the raw and imputed sample types, and after adjusting our significance criterion for multiple testing, the SNP's effects were no longer significant for the imputed samples. Although these two approaches differed in their aims—the gene-based approach ignored unique effects of the SNPs in favor of examining common variation, whereas the SNP-based approach ignored common variation among the SNPs in favor of examining unique effects—if we were able to choose only one of these methods to examine a novel genetic association, it seems that the gene-based approach would provide the more robust and powerful test of the gene's effects overall.

We did not detect any sex differences in the associations between *AVPR1A* and aggression in the present study. This was surprising given findings from previous studies that have suggested the effects of AVP on aggression are sex- and/or hormone-dependent (Nephew & Bridges, 2008; Nephew et al., 2010). Because our test of the *AVPR1A* x sex interaction may not have been powerful enough to detect the presence of small sex differences in the hypothesized associations, we chose to conduct several post-hoc analyses that would allow us to contrast the regression coefficients for the association between the *AVPR1A* factor and aggression separately for males and females. Due to the complexity of the full model, it was necessary to make several minor changes to the model (i.e., removing ancestry as a covariate and collapsing across heterozygous and homozygous categories for the minor allele of two SNPs with low MAFs, rs11174803 and rs11174820) in order to produce results separately for males and females. The results of these post-hoc analyses revealed a significant association between the *AVPR1A* factor and aggression for girls that contributed a substantial proportion of the variance in aggression, $B = -.031$, $SE = .073$, $z = -4.23$, $p < .001$, $R^2 = .12$. In contrast, no such association was found for boys

B = -.085, *SE* = .070, *z* = 1.20, *p* = .227, R^2 = .006. These findings suggest that there may indeed be sex differences in the effects of *AVPR1A*, and that this gene may play a particularly important role in female aggression. Nonetheless, it will be important for future studies to replicate this finding using larger samples, which may allow investigators to control for participant ancestry and thus reduce the possibility that such findings may be due to the effects of population stratification.

With respect to the nonsignificance of the *AVPR1A* x sex interaction term, it is also plausible that sexual dimorphism for *AVPR1A*'s effects is not present until mid- to late-adolescence. Because our sample comprised children and adolescents who ranged in age from 6-18 years in age, we would have had difficulty detecting sex differences that develop later in adolescence. We were unable to conduct separate tests of this interaction for the children and adolescents due to the size of our sample and the complexity of these models, but future studies should consider conducting these tests stratified by age group.

There were some limitations to the current study. Primarily, because we utilized a gene-based approach for testing association, we were unable to infer the specific nature of the associations between *AVPR1A* and aggression. Although we found that variation within this gene was associated with variation in aggressive behavior, these tests did not indicate which allele(s) were responsible for these associations. Because this method is meant to serve as an initial test of genetic association, one that may be replicated across samples even in the presence of different genotyped SNPs and/or differences in LD without the need for a myriad of separate statistical tests, it is not designed to provide specific information regarding the causal variation within the gene. Nonetheless, even SNP-based tests typically fail to meet this standard; we cannot infer from a SNP-based test that an allele that shows significant association is a causal variant, we can only infer that it may be *associated* with a causal variant through LD. In

order to understand the source(s) of causal variation within *AVPR1A* (following replications of the gene-based effect, of course), one might choose to separate the gene into several regions for further analysis (e.g., by using exploratory factor analysis to identify haplotype blocks within the gene that may differentially contribute to common variation in aggression). Using such a method, one might successively narrow down the region(s) of the gene responsible for producing the differences in receptor morphology or function driving the association.

A second limitation was sample size. Although there were over six hundred children genotyped for the current investigation, over one fourth of these individuals (who were gathered from various sources, as noted in the Methods) did not have the appropriate phenotypic information available. The reduction in statistical power resulting from this missing phenotypic information may have limited our ability to detect sex differences in the magnitude of the reported associations between *AVPR1A* and aggression. Because genetic association studies typically yield very small main effects and interactive effects are even more difficult to detect than main effects (McClelland & Judd, 1993), it will be important for future studies of this gene to utilize very large samples.

The current investigation was the first to utilize a latent variable model gene-based test to examine associations between AVP-ergic system genes and aggression. Further, it was the first to our knowledge to examine the role of the *AVPR1A* receptor gene in child and adolescent aggression. Our findings suggest that common variation in this gene is associated with variation in aggression in our sample. In order to better understand how variation in this gene influences aggressive outcomes, future studies should continue to examine the *AVPR1A* gene and its role in AVP-ergic functioning.

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Table 1

Sample attributes (children only)

	Genotyped *N*	Phenotypic *N* (Aggression/Sociability)	Age *M* (*SD*)	% Male
Full sample	621	450/434	11.56 (3.48)	62.8%
High-quality subsample	530	389/375	11.69 (3.51)	60.6%
High-quality subsample, European ancestry	454	358/350	11.81 (3.48)	60.6%
Imputed sample	621	450/434	11.56 (3.48)	62.8%
Imputed high-quality subsample	530	389/375	11.69 (3.51)	60.6%

Table 2

Results of genotyping quality control analyses: Full Sample

SNP	Call Rate	MZ Discord. Rate	Genotype Frequencies			HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	MAF	HapMap MAF (CEU)	HapMap MAF (YRI)
						All Individuals		Unrelateds		Founders				
			CC	**CG**	**GG**									
rs11174803	94.9%	2.63%	.02	.21	.77	2.36	.139	.26	.560	.71	.361	.125	.129	NA
			AA	**GA**	**GG**									
rs962862	85.1%	0.00%	.01	.09	.90	11.74	.003*	8.17	.019	8.32	.015	.056	.049	.007
			CC	**TC**	**TT**									
rs1587097	81.7%	5.71%	.84	.14	.02	30.36	2e-06*	13.81	.001*	6.83	.016	.092	.086	.003
			CC	**CG**	**GG**									
rs2738250	83.3%	2.78%	.82	.15	.02	22.29	6e-05*	2.35	.132	3.06	.089	.103	.093	.000
			CC	**CG**	**GG**									
rs4763062	83.7%	5.41%	.02	.17	.81	4.69	.039	5.94	.031	4.76	.012	.103	.198	.087
			GG	**GT**	**TT**									
rs11174808	80.7%	0.00%	.01	.01	.98	239.47	6e-09*	113.73	2e-05*	136.18	7e-07*	.010	.009	.007
			CC	**CT**	**TT**									
rs11174820	80.9%	2.94%	.94	.06	<.01	3.23	.099	2.66	.143	3.34	.117	.034	.108	.000
			AA	**GA**	**GG**									
rs7307997	77.6%	0.00%	.35	.42	.23	18.45	2e-05*	5.16	.022	8.07	.005*	.438	.408	.111

Note: MZ discordance rate calculated only for MZ pairs in which genotyping was completed for both individuals within the pair; HWE *p* statistic provided by Exact test of Hardy-Weinberg Equilibrium in Pedstats; SNP indicates single nucleotide polymorphism examined; Discord. Indicates discordance; HWE indicates Hardy-Weinberg Equilibrium; MAF indicates minor allele frequency; CEU indicates individuals of European ancestry; YRI indicates individuals of African ancestry.

Table 3

Results of genotyping quality control analyses: High-Quality Subsample

SNP	Call Rate	MZ Discord. Rate	Genotype Frequencies			HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	MAF	HapMap MAF (CEU)	HapMap MAF (YRI)
						All Individuals		Unrelateds		Founders				
			CC	**CG**	**GG**									
rs11174803	96.7%	2.94%	.02	.22	.77	.48	.469	.00	>.999	.07	>.999	.126	.129	NA
			AA	**GA**	**GG**									
rs962862	99.2%	0.00%	.01	.09	.90	12.35	3e-03*	8.06	.020	8.94	.012	.056	.049	.007
			CC	**TC**	**TT**									
rs1587097	97.0%	6.06%	.84	.14	.02	27.67	4e-06*	13.75	.001*	7.11	.014	.092	.086	.003
			CC	**CG**	**GG**									
rs2738250	98.5%	2.86%	.82	.16	.02	17.56	1e-04*	2.31	.134	2.92	.093	.101	.093	.000
			CC	**CG**	**GG**									
rs4763062	98.9%	2.86%	.02	.17	.81	3.72	.058	5.90	.031	7.81	.012	.102	.198	.087
			GG	**GT**	**TT**									
rs11174808	96.0%	0.00%	<.01	.01	.99	25.29	.034	27.57	.030	22.55	.037	.006	.009	.007
			CC	**CT**	**TT**									
rs11174820	94.7%	3.03%	.93	.06	<.01	.02	>.999	.31	.467	.36	.433	.033	.108	.000
			AA	**GA**	**GG**									
rs7307997	92.8%	0.00%	.35	.42	.22	17.71	3e-05*	5.16	.022	7.89	.005*	.439	.408	.111

Note: MZ discordance rate calculated only for MZ pairs in which genotyping was completed for both individuals within the pair; HWE *p* statistic provided by Exact test of Hardy-Weinberg Equilibrium in Pedstats; SNP indicates single nucleotide polymorphism examined; Discord. Indicates discordance; HWE indicates Hardy-Weinberg Equilibrium; MAF indicates minor allele frequency; CEU indicates individuals of European ancestry; YRI indicates individuals of African ancestry.

Table 4

Results of genotyping quality control analyses: High-Quality Subsample, European Ancestry

SNP	Call Rate	MZ Discord. Rate	Genotype Frequencies			HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	MAF	HapMap MAF (CEU)	HapMap MAF (YRI)
						All Individuals		Unrelateds		Founders				
			CC	**CG**	**GG**									
rs11174803	96.8%	2.94%	.02	.22	.77	.77	.375	.05	.833	.02	>.999	.128	.129	NA
			AA	**GA**	**GG**									
rs962862	99.3%	0.00%	.01	.10	.89	12.19	3e-03*	8.41	.018	9.24	.011	.058	.049	.007
			CC	**TC**	**TT**									
rs1587097	96.8%	6.06%	.84	.14	.02	27.34	5e-06*	13.93	.001*	7.38	.013	.095	.086	.003
			CC	**CG**	**GG**									
rs2738250	98.4%	2.86%	.82	.16	.02	16.62	2e-04*	2.00	.147	2.57	.105	.105	.093	.000
			CC	**CG**	**GG**									
rs4763062	98.9%	2.86%	.02	.17	.81	4.23	.051	6.55	.016	8.79	.010	.104	.198	.087
			GG	**GT**	**TT**									
rs11174808	96.3%	0.00%	<.01	.01	.99	28.56	.030	34.08	.024	27.26	.030	.006	.009	.007
			CC	**CT**	**TT**									
rs11174820	94.4%	3.03%	.93	.07	<.01	.01	>.999	.23	.470	.36	.433	.035	.108	.000
			AA	**GA**	**GG**									
rs7307997	92.6%	0.00%	.33	.43	.24	14.11	2e-04*	3.22	.078	5.69	.017	.453	.408	.111

Note: MZ discordance rate calculated only for MZ pairs in which genotyping was completed for both individuals within the pair; HWE *p* statistic provided by Exact test of Hardy-Weinberg Equilibrium in Pedstats; SNP indicates single nucleotide polymorphism examined; Discord. Indicates discordance; HWE indicates Hardy-Weinberg Equilibrium; MAF indicates minor allele frequency; CEU indicates individuals of European ancestry; YRI indicates individuals of African ancestry.

Table 5

Results of genotyping quality control analyses: Imputed Full Sample

SNP	R^2 IMP	MZ Discord. Rate	Genotype Frequencies			HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	MAF	HapMap MAF (CEU)	HapMap MAF (YRI)
						All Individuals		Unrelateds		Founders				
			CC	**CG**	**GG**									
rs11174803	0.97	4.65%	.02	.21	.77	2.83	.111	.80	.356	.90	.345	.125	.129	NA
			AA	**GA**	**GG**									
rs962862	0.86	2.33%	.01	.08	.91	27.85	3e-05*	25.38	1e-04*	26.3	9e-05*	.050	.049	.007
			CC	**TC**	**TT**									
rs1587097	0.91	6.98%	.82	.16	.02	11.42	.002*	2.18	.161	1.35	.238	.100	.086	.003
			CC	**CG**	**GG**									
rs2738250	0.76	11.63%	.69	.29	.02	1.72	.213	3.54	.075	3.56	.074	.165	.093	.000
			CC	**CG**	**GG**									
rs4763062	0.74	11.63%	.01	.30	.68	11.29	5e-04*	4.18	.047	3.90	.061	.162	.198	.087
			GG	**GT**	**TT**									
rs11174808	0.74	2.33%	<.01	.01	.99	296.73	2e-09*	170.04	3e-07*	163.21	4e-07*	.005	.009	.007
			CC	**CT**	**TT**									
rs11174820	0.82	9.30%	.95	.05	<.01	4.88	.060	5.73	.066	5.39	.071	.025	.108	.000
			AA	**GA**	**GG**									
rs7307997	0.79	13.95%	.28	.54	.18	9.42	.003*	6.06	.017	5.58	.023	.450	.408	.111

Note: MZ discordance rate calculated only for MZ pairs in which genotyping was completed for both individuals within the pair; HWE *p* statistic provided by Exact test of Hardy-Weinberg Equilibrium in Pedstats; SNP indicates single nucleotide polymorphism examined; Discord. Indicates discordance; HWE indicates Hardy-Weinberg Equilibrium; MAF indicates minor allele frequency; CEU indicates individuals of European ancestry; YRI indicates individuals of African ancestry.

Table 6

Results of genotyping quality control analyses: Imputed High-Quality Subsample

SNP	R^2 IMP	MZ Discord. Rate	Genotype Frequencies			HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	HWE χ^2	HWE *p*	MAF	HapMap MAF (CEU)	HapMap MAF (YRI)
						All Individuals		Unrelateds		Founders				
			CC	**CG**	**GG**									
rs11174803	0.99	5.71%	.02	.22	.76	.56	.478	.10	1.00	.07	1.00	.130	.129	NA
			AA	**GA**	**GG**									
rs962862	0.99	0.00%	.01	.09	.90	12.08	.003*	8.47	.014	8.93	.012	.055	.049	.007
			CC	**TC**	**TT**									
rs1587097	0.97	8.57%	.82	.16	.02	15.98	4e-04*	3.74	.061	2.41	.166	.100	.086	.003
			CC	**CG**	**GG**									
rs2738250	0.96	2.86%	.81	.17	.02	13.6	6e-04*	1.81	.185	1.85	.183	.105	.093	.000
			CC	**CG**	**GG**									
rs4763062	0.97	2.86%	.02	.18	.81	2.65	.129*	4.84	.038	6.03	.019	.109	.198	.087
			GG	**GT**	**TT**									
rs11174808	0.93	2.86%	<.01	.01	.99	26.38	.032	19.22	.043	22.11	.037	.001	.009	.007
			CC	**CT**	**TT**									
rs11174820	0.93	8.57%	.94	.06	<.01	.00	1.00	.46	.408	.53	.394	.001	.108	.000
			AA	**GA**	**GG**									
rs7307997	0.94	0.00%	.33	.46	.21	5.13	.025	2. 42	.152	2.72	.113	.440	.408	.111

Note: MZ discordance rate calculated only for MZ pairs in which genotyping was completed for both individuals within the pair; HWE *p* statistic provided by Exact test of Hardy-Weinberg Equilibrium in Pedstats; SNP indicates single nucleotide polymorphism examined; Discord. Indicates discordance; HWE indicates Hardy-Weinberg Equilibrium; MAF indicates minor allele frequency; CEU indicates individuals of European ancestry; YRI indicates individuals of African ancestry.

Table 7

Model results for gene-based tests of AVPR1A and aggression

	χ^2	*p*	N	RMSEA	CFI	TLI	WRMR	AIC	BIC	*Coefficient*	*SE*	*Z* (reg.)	*p*
MEASUREMENT MODELS													
AGG only	162.58	<.01	471	.065	.99	.98	.90	282.58	531.87				
AGG with all covariates[1]	193.6	<.01	452	.028	.99	.99	.84	329.62	609.35				
AGG with reduced covariates	211.7	<.01	452	.051	.99	.98	.94	339.78	603.06				
AVPR1A with 8 SNPs	335.0	<.01	623	.159	.68	.55	2.83	383.00	489.43				
AVPR1A with 7 SNPs	203.4	<.01	623	.147	.70	.55	2.33	245.40	338.53				
AVPR1A with 6 SNPs	86.2	<.01	623	.117	.88	.79	1.65	122.19	202.01				
STRUCTURAL MODELS													
Raw Data													
Full sample													
AGG on AVPR1A (8 SNPs)	542.5	<.01	573	0.046	0.965	0.961	1.39	720.48	1108.01	-0.094	0.042	-2.25	0.02*
AGG on AVPR1A (7 SNPs)	446.3	<.01	573	0.042	0.974	0.971	1.24	618.30	992.77	-0.113	0.058	-1.96	0.05*
AGG on AVPR1A (6 SNPs)	353.6	<.01	573	0.036	0.982	0.98	1.09	519.56	880.97	-0.106	0.059	-1.78	0.08[ǂ]

High-quality subsample													
AGG on AVPR1A (8 SNPs)	516.7	<.01	489	0.048	0.964	0.96	1.348	690.67	1055.41	-0.112	0.059	-1.92	0.06[ŧ]
AGG on AVPR1A (7 SNPs)	427.0	<.01	489	0.043	0.973	0.97	1.209	596.98	953.33	-0.113	0.058	-1.94	0.05*
AGG on AVPR1A (6 SNPs)	339.1	<.01	489	0.037	0.982	0.98	1.06	503.06	846.83	-0.103	0.060	-1.73	0.08[ŧ]
Imputed Data													
Full sample													
AGG on AVPR1A (8 SNPs)	576.7	<.01	573	0.049	0.961	0.96	1.44	754.70	1141.92	-0.122	0.048	-2.58	0.01*
AGG on AVPR1A (7 SNPs)	499.3	<.01	573	0.047	0.967	0.96	1.34	671.33	1045.50	-0.130	0.055	-2.37	0.02*
AGG on AVPR1A (6 SNPs)	408.4	<.01	573	0.042	0.976	0.97	1.20	574.40	935.52	-0.122	0.056	-2.19	0.03*
High-quality subsample													
AGG on AVPR1A (8 SNPs)	482.3	<.01	489	0.045	0.968	0.965	1.29	656.26	1020.82	-0.119	0.059	-2.03	0.04*
AGG on AVPR1A (7 SNPs)	463.9	<.01	489	0.044	0.972	0.965	1.23	633.86	990.04	-0.115	0.059	-1.95	0.05*
AGG on AVPR1A (6 SNPs)	338.9	<.01	489	0.037	0.982	0.98	1.06	502.89	846.50	-0.099	0.060	-1.64	0.10[ŧ]

Note: [1]Despite favorable fit statistics, covariances within the measurement model of aggression that included all covariates indicated strong multicollinearity among predictors, so a reduced number of covariates was selected for inclusion in the following structural models; AGG indicates the latent aggression factor; *indicates significance of the regression coefficient at .05 level; [ŧ]indicates marginal significance at .10 level

Table 8

Model results for SNP-based tests of AVPR1A and aggression

	χ^2	*p*	N	RMSEA	CFI	TLI	WRMR	AIC	BIC	*Coefficient*	*SE*	*Z* (reg.)	*p*
Raw Data													
Full sample/	233.11	.01	308	.029	.99	.99	.76	377.11	645.68				
High-quality													
subsample[a]													
rs11174803										-.827	.279	-2.96	.003*
rs962862										.540	.240	2.25	.02
rs1587097										.618	.297	2.08	.04
rs2738250										.057	.200	.29	.78
rs4763062										.089	.149	.60	.55
rs11174808										-.741	1.045	-.71	.48
rs11174820										.018	.248	.07	.94
rs7307997										.144	.147	.98	.33
Imputed Data													

Full sample	264.15	<.01	450	.031	.99	.99	.83	408.15	704.02				
rs11174803										-.508	.229	-2.22	.03
rs962862										.209	.187	1.12	.26
rs1587097										.379	.248	1.53	.13
rs2738250										.160	.129	-1.24	.22
rs4763062										.061	.117	.52	.60
rs11174808										.021	.485	-.04	.97
rs11174820										.103	.211	.49	.63
rs7307997										.125	.123	1.02	.31
High-quality subsample	241.66	<.01	389	.028	.99	.99	.77	385.66	671.03				
rs11174803										-.533	.228	-2.34	.02
rs962862										.350	.227	1.69	.09
rs1587097										.368	.248	1.49	.14
rs2738250										-.068	.157	-.44	.66
rs4763062										.110	.140	.79	.43

rs11174808	.664	.964	-.69	.49
rs11174820	.101	.219	.46	.64
rs7307997	.102	.128	.80	.43

Note: covariates included in regression analysis include proportion European ancestry, sex, age, age^2; * indicates significance at p = .00625 level; [a]because models were estimated based on individuals who had information available for all predictor variables, fit statistics and parameter estimates from the SNP-based tests were identical for the full sample and high-quality subsample within the raw dataset.

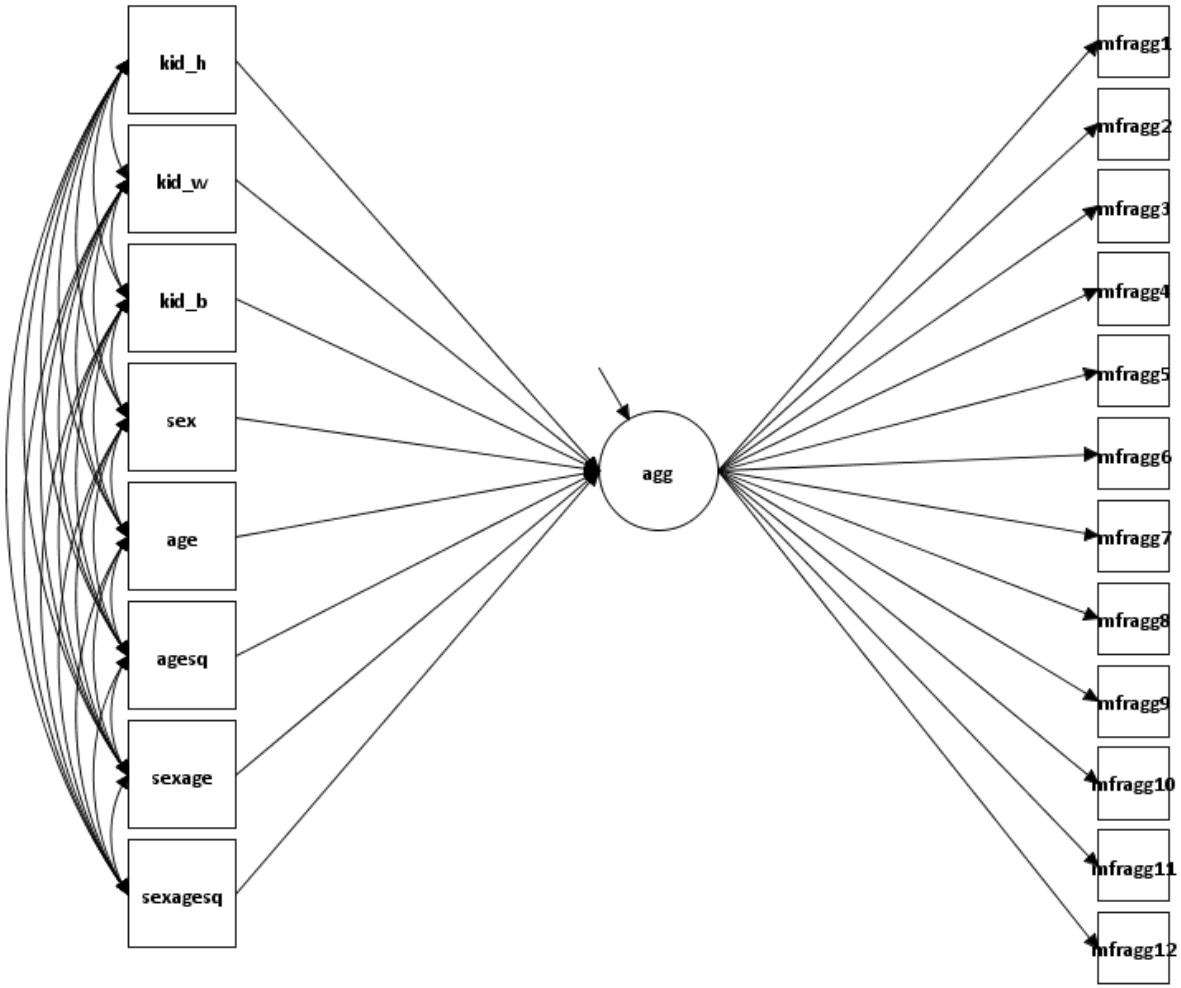

Figure 1. Latent aggression factor with all covariates. Covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From top to bottom, covariates include Hispanic ancestry, European ancestry, African ancestry, sex, age, age^2, sex X age interaction term, sex X age^2 interaction term.

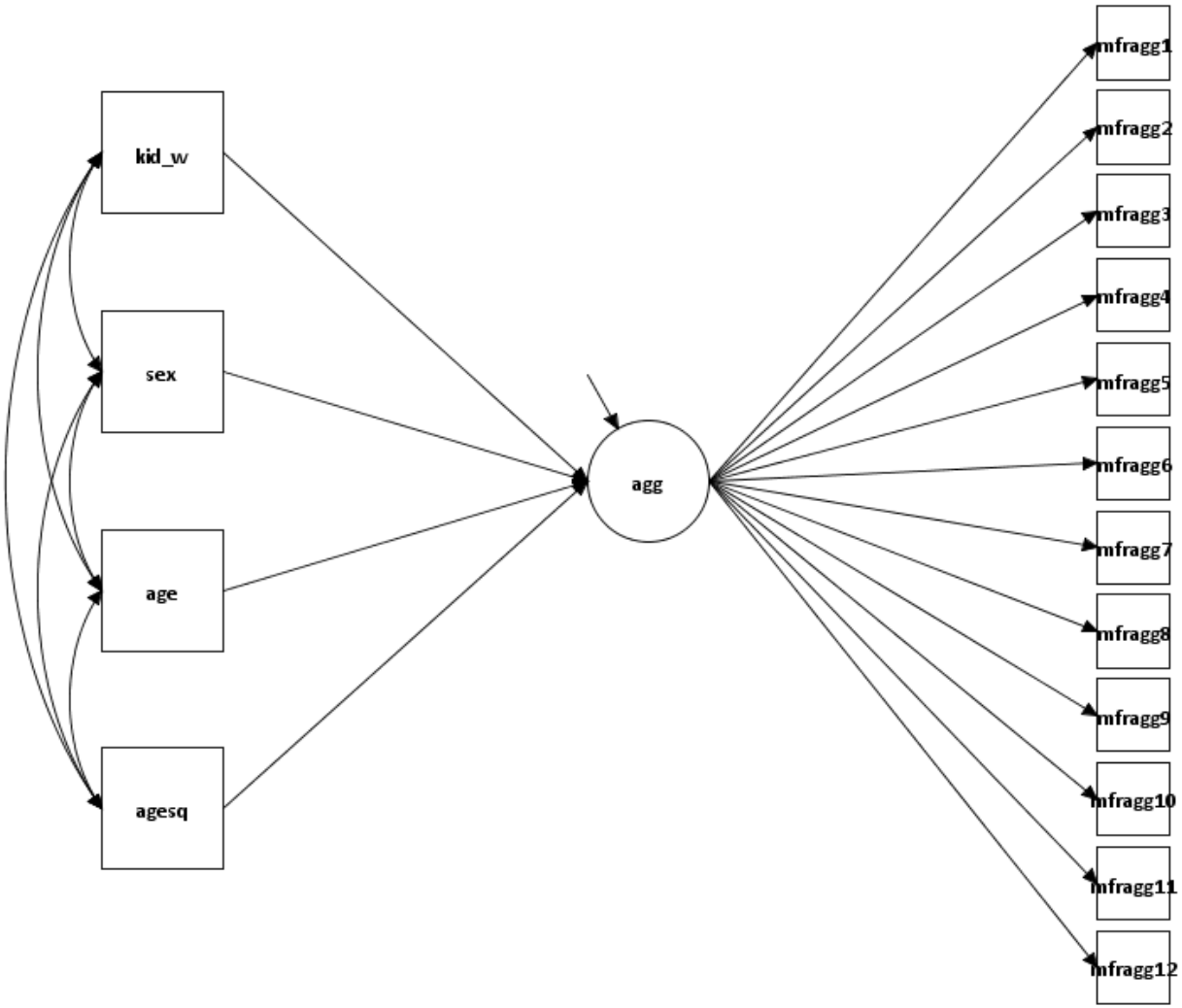

Figure 2. Latent aggression factor with reduced covariates. Covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

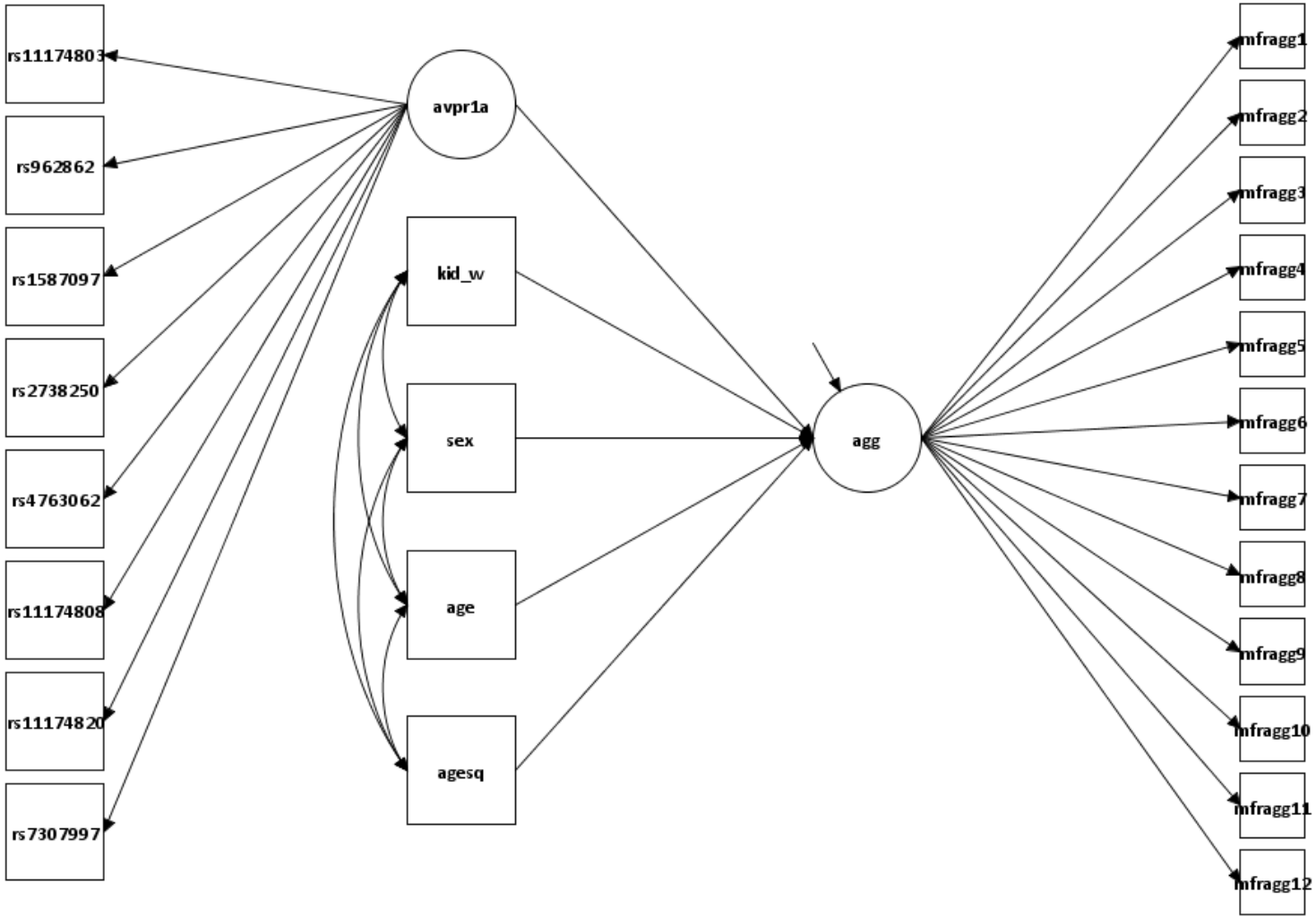

Figure 3. Latent aggression factor with reduced covariates and *AVPR1A* (8 SNPs). Covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

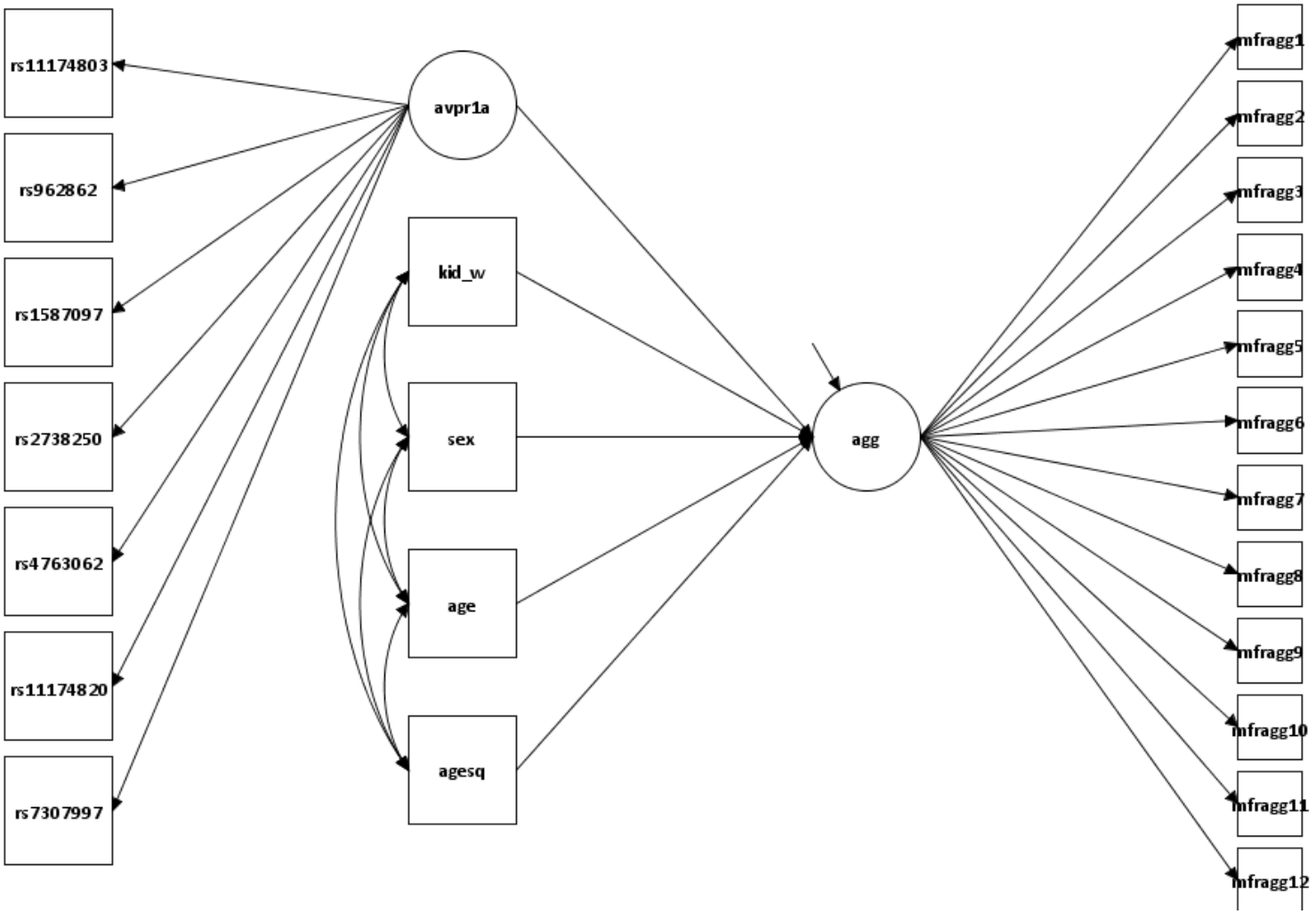

Figure 4. Latent aggression factor with reduced covariates and *AVPR1A* (7 SNPs). Covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

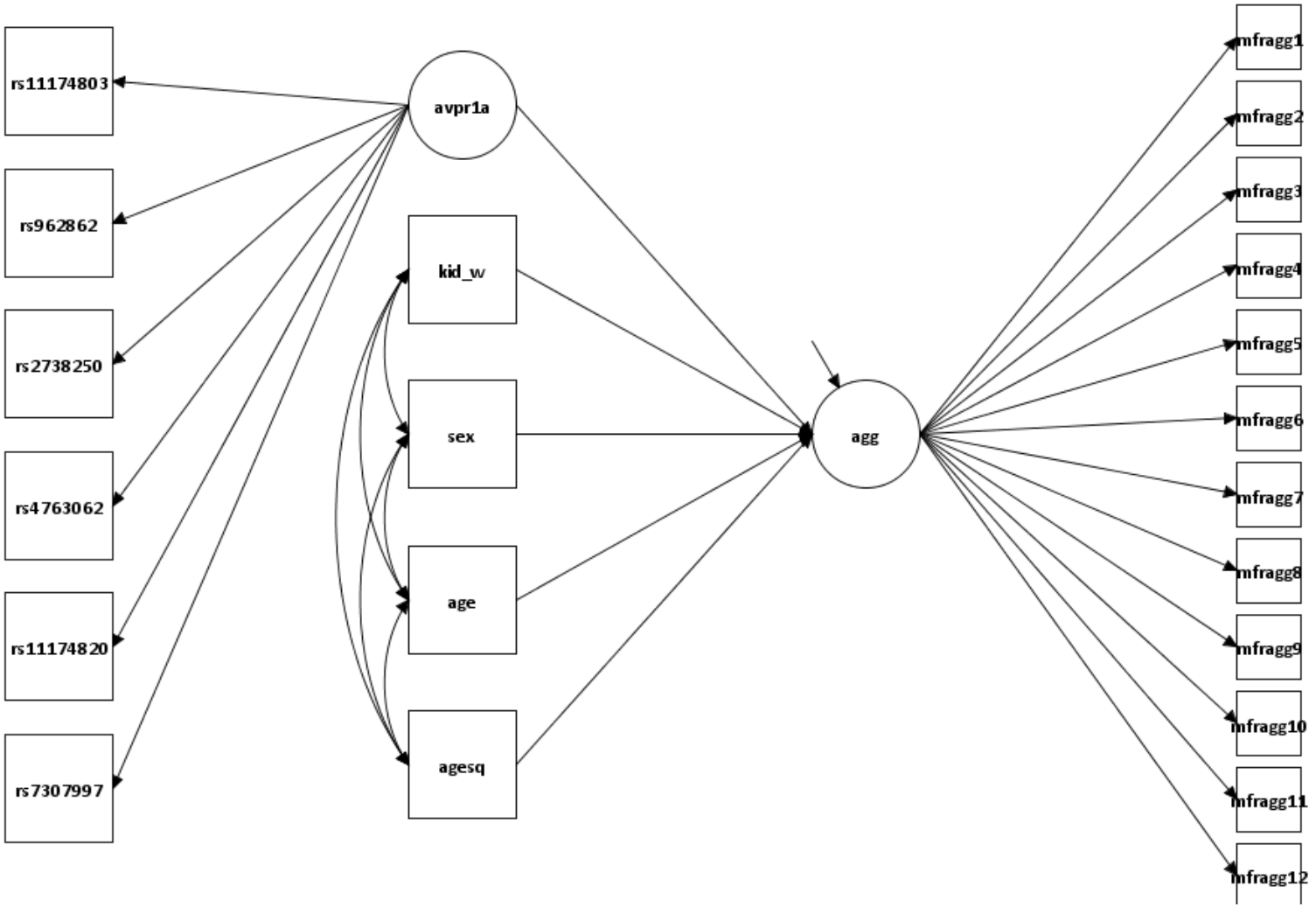

Figure 5. Latent aggression factor with reduced covariates and *AVPR1A* (6 SNPs). Covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

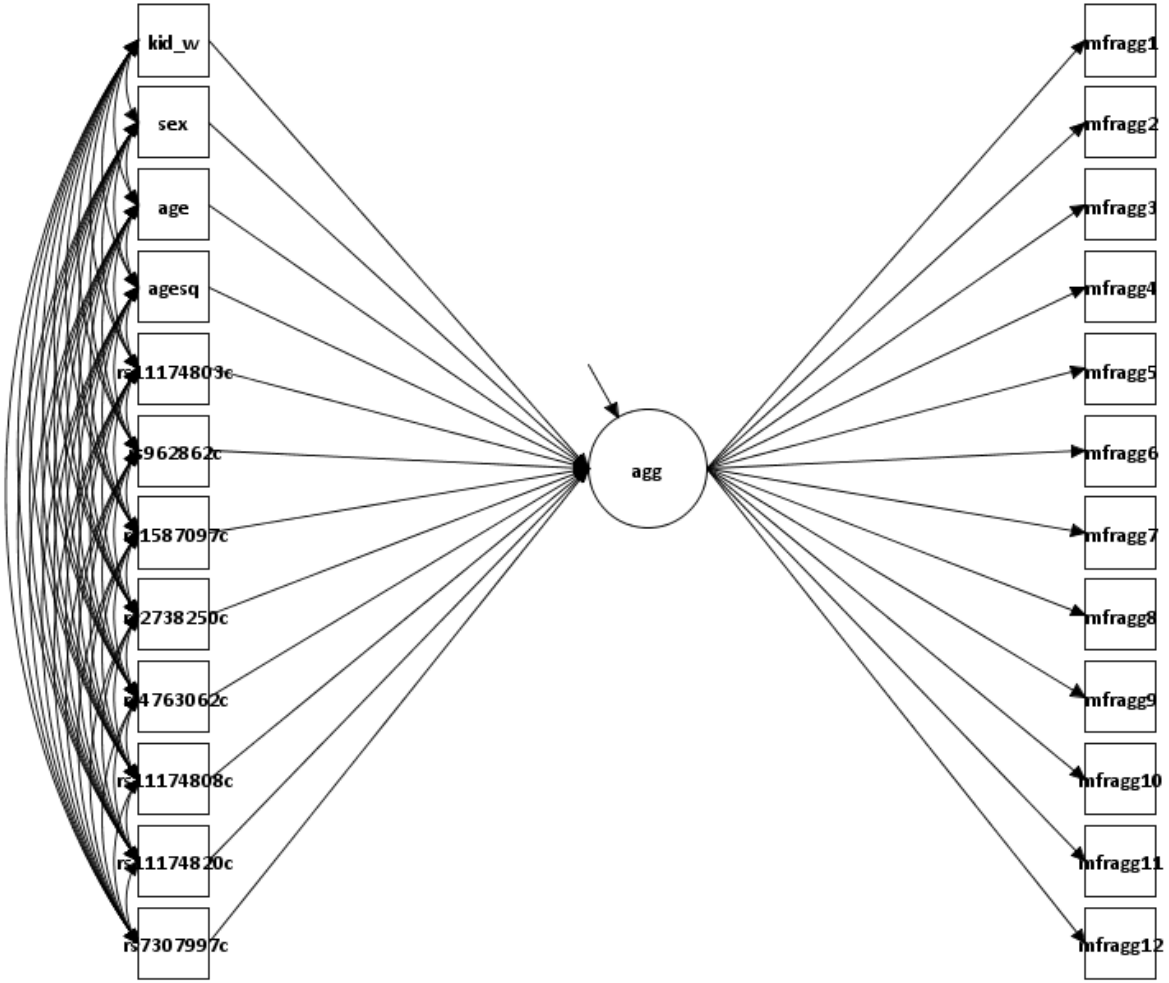

Figure 6. SNPs-only test of latent aggression factor and *AVPR1A*. SNPs and covariates are on the left side of the latent aggression factor; factor indicators (Dodge & Coie items, mfragg1-12) are on the right side. From the top, covariates European ancestry, sex, age, and age^2. The "c" following each SNP rs number indicates the SNP was coded as a binary categorical variable.

AVPR1A and Prosocial Behavior

Courtney A. Ficks and Irwin D. Waldman

Emory University

Abstract

Vasopressin (AVP) has been shown to play a role in normative social behaviors such as social recognition and bonding in nonhuman animals. Studies have recently begun to examine the impact of genetic variation within the AVP-ergic system, particularly for the AVPR1a receptor gene (*AVPR1A*), on related social phenotypes in humans such as prosocial behavior. Nonetheless, only a handful of such studies have been conducted thus far, and findings regarding the effects of specific markers have been inconsistent across studies. The present investigation examined the effects of *AVPR1A* on sociability, a dimension of child temperament related to prosocial behavior, using a gene-based, latent variable approach that was previously utilized by the investigators of this study in the examination of childhood aggression. Six hundred and twenty-one children ages 6-18 years were genotyped for 8 single nucleotide polymorphisms (SNPs) in *AVPR1A*, and parent reports of the children's sociability were obtained using the EAS Temperament Survey (Buss & Plomin, 1984). Genotyping quality for this sample was rigorously assessed in a previous investigation (see Study 1). Sociability was modeled as a single latent factor indicated by both mother- and father-reported sociability items using a confirmatory factor analytic approach. *AVPR1A* was also modeled as a latent genetic factor indicated by the categorically-coded genotyped SNPs. Sociability was regressed on the latent genetic factor in addition to several covariates (i.e, age, sex, age^2, and ethnicity). Results suggested significant genetic associations for two models, but further inspection of these associations revealed these associations to be driven by a single SNP with very low minor allele frequency. No significant associations were present in any of the subsequent models, suggesting that *AVPR1A* did not contribute to variation in children's sociability in the current sample. Implications regarding the use of this gene-based, latent variable approach as well as the examination of prosocial phenotypes in future studies of *AVPR1A* were discussed.

AVPR1A and Prosocial Behavior

The term "prosocial" has been used to describe social behavior that is "intended to benefit one or more people other than oneself . . . such as helping, comforting, sharing, and cooperating" (Batson & Powell, 2003) and includes actions involved in the formation and maintenance of social relationships. Although this term is sometimes used as an antonym for "antisocial," (Batson & Powell, 2003), there is evidence to suggest that prosocial behaviors exist along a separate dimension from antisocial behaviors (Krueger, Hicks, & McGue, 2001) and are etiologically distinct (Krueger et al., 2001). Indices of prosocial behavior, including altruism, empathy, and nurturance, have demonstrated moderate to high heritability (Knafo & Plomin, 2006; Penner, Dovidio, Piliavin, & Schroeder, 2005; Rushton, Fulker, Neale, Nias, & Eysenck, 1986), but few studies have explored the genetic influences underlying these behaviors (e.g., Jiang, Chew, & Ebstein, 2013; Knafo, Israel, & Ebstein, 2011). Prosocial behavior has been shown to predict children's social adjustment (Crick, 1996) and responses to peer victimization (Griese & Buhs, 2013) and thus may represent an important protective factor during development.

Despite growing evidence for AVP's roles in prosocial behavior (e.g., Bielsky & Young, 2004; Guastella, Kenyon, Alvares, Carson, & Hickie, 2010; Young, Gobrogge, Liu, & Wang, 2011; Zink et al., 2011), only a handful of studies have examined associations between variation in the *AVPR1A* gene and prosocial behavior in humans. In one study, Knafo et al. (2008) tested whether *AVPR1A* was associated with altruistic behavior in a game that involved the allocation of funds to other players. The authors reported that the RS3 microsatellite (discussed previously) was associated with altruism in that men and women homozygous for "long" repeat lengths (327-343 bp) allocated more funds and rated themselves as significantly more altruistic than individuals homozygous for "short" repeat lengths (308-325 bp) (Knafo et al., 2008). No significant associations were reported for the RS1 microsatellite. Using a modified version of this

paradigm in which preschoolers were allowed to allocate stickers to themselves and an unknown partner, Avinun et al. (2011) found that individuals with at least one copy of the 327 bp allele exhibited significantly less altruism than their peers (Avinun et al., 2011). Because these investigators utilized a twin sample, both population-based and family-based analyses were conducted in order to rule out potential confounders within the putative associations, and across both analytic methods findings were consistent (Avinun et al., 2011). Nonetheless, because the "long" repeat length containing this allele as reported by Knafo et al. (2008) was previously associated with *increased* altruism in an adult sample, overall findings for the role of the 327 bp allele in altruistic behavior appear inconsistent.

Following evidence for the role of AVP in pair bonding (see Young et al., 2011), several studies have specifically examined the role of *AVPR1A* in romantic relationships. Although a genome-wide linkage and association study did not detect a significant association between RS3 and infidelity or number of sexual partners in a sample of women (Cherkas, Oelsner, Mak, Valdes, & Spector, 2004), Walum et al. (2008) reported a significant association between the 334 bp allele of RS3 and monogamous behavior in men. Cohabiting men carrying the 334 bp allele were more likely to report relationship problems, reduced bonding with their partner, and were less likely to be married to their cohabiting partner (Walum et al., 2008). In addition, women cohabiting with male carriers reported lower relationship quality across various indices (Walum et al., 2008). In support of Cherkas et al. (2004), there were no associations between RS3 genotype and monogamous behavior in women and no associations between RS1 or a third GT25 microsatellite and either males' or females' behavior. Clearly, more research is needed to understand how variation in this gene contributes to individual differences in prosocial behaviors. Further, it is important that we test for sex differences in these associations, as there is some evidence to suggest that findings may differ for males and females.

As there is little evidence regarding the role of *AVPR1A* in prosocial behaviors, we may refer to studies of another phenotype, autism spectrum disorders (ASDs, which are often characterized by impairment in affiliative social behavior; Yrigollen et al., 2008), for additional clues about how this gene may be involved in the ability to form and maintain social relationships. Several investigations have examined associations between *AVPR1A* variants and ASDs (with a primary focus on the aforementioned RS1 and RS3 repeat sequences). Kim et al. (2002) reported significant overtransmission of the 340 bp allele of RS3 in individuals diagnosed with autism, but associations were not significant after correcting for multiple testing (Kim et al., 2002). The authors did not report any significant associations between RS1 transmission and autism diagnoses (Kim et al., 2002). In contrast, another study found evidence for the overtransmission of the 328 bp allele in RS3 and the 320 bp allele of RS1 and the undertransmission of the 312 bp allele of RS1 within families of more highly functioning individuals with autism (i.e., those without language impairment) (Wassink et al., 2004). To further complicate matters, Yirmiya et al. (2006) reported no associations between RS1 or RS3 and autism diagnoses, but an omnibus test of a separate intronic microsatellite AVR revealed significant transmission disequilibrium for AVR (Yirmiya et al., 2006). A haplotype analysis of all three microsatellites (RS1, RS3, and AVR) revealed significant transmission disequilibrium for 7 haplotypes, with the most common haplotype showing overtransmission of the 212 bp, 314 bp, and 325 bp alleles of AVR, RS1, and RS3, respectively, in individuals with autism (Yirmiya et al., 2006).

Tansey and colleagues (2011) recently found associations between “short” RS1 alleles (310 bp or fewer) and autism but did not find any associations with RS3 or AVR (Tansey et al., 2011). This study also tagged four single nucleotide polymorphisms (SNPs) within *AVPR1A* and reported significant undertransmission of the A allele of rs11174815 in individuals with autism

(Tansey et al., 2011). Nonetheless, because the undertransmitted allele had a very low minor allele frequency (0.015), the investigators were skeptical regarding the robustness of this finding (Tansey et al., 2011).

A recent meta-analysis of findings from these studies examined 5 SNPs within RS1 and 6 SNPs within RS3 and found that none of these SNPs exhibited significant effects on ASDs across studies (LoParo, 2013). Further, classifying RS1 and RS3 alleles as "long" and "short" also did not yield significant associations across studies (LoParo, 2013). Nonetheless, because this meta-analysis only included data from four independent samples (with a total of 486 participants), it is important that we continue to examine associations between AVPR1A and ASDs using large, representative samples before drawing strong conclusions about these findings. Overall, although several studies have reported significant associations between variation in *AVPR1A* and ASDs, which may support a role for this gene in prosocial behavior, the specific nature of these associations remains unclear due to the small number of available studies and inconsistencies in the variants implicated in these disorders across studies.

The current study.

We attempted to overcome weaknesses in previous studies while extending the extant literature in several ways. First, as in Study 1, we conducted gene-based tests of the effects of *AVPR1A* on our phenotype of interest, sociability, by modeling the gene as a latent variable indicated by 8 genotyped SNPs across the gene. This method was considered a better alternative to traditional tests of association for two (related) reasons: 1) it allowed us to utilize a single omnibus statistical test of association, eliminating the need to conduct separate tests for each SNP (which would inflate the probability of Type I error given multiple testing), and 2) specifying a latent genetic factor to represent variation in the *AVPR1A* SNPs allowed us to

exploit the pattern of LD (correlations among SNPs) across the gene and to test for the effects of this common variation on our phenotype of interest.

Another strength of this study was its focus on a normative, continuously-distributed, and theoretically relevant social phenotype: *sociability*. Given the instrument that we used (see Methods), *sociability* in the current study may be conceptualized as an aspect of temperament that encompasses one's interest and enjoyment in spending time with others. Children's sociability scores have been shown to predict their social skills, interactions, and relationships with peers and adults (Sanson, Hemphill, & Smart, 2004). This phenotype is highly appropriate for genetic research because sociability has demonstrated good temporal stability (Mathiesen & Tambs, 1999) and appears to have a substantial genetic basis (Benish-Weisman, Steinberg, & Knafo, 2010). As noted previously, despite the number of studies implicating AVP in prosocial behavior in humans and nonhuman animals, few studies have actually examined the effects of *AVPR1A* on variation in prosocial behaviors in normative populations (Avinun et al., 2011; Cherkas et al., 2004; Knafo et al., 2008; Walum et al., 2008) and only one study has examined its effects on prosocial behaviors in children (Avinun et al., 2011). The examination of socially-relevant phenotypes such as sociability that are continuously distributed in the general population (as opposed to positively-skewed ASD traits or categorical ASD diagnoses) allows us greater power to detect statistical associations that may be present.

As a second aim of the current study, we tested an *AVPR1A* x sex interaction term within the proposed latent variable model. Because there is evidence that AVP's influence on prosocial behavior may be sexually dimorphic in both human (Cherkas et al., 2004; Walum et al., 2008) and nonhuman species (e.g., Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012; Insel & Hulihan, 1995), we felt that it was important to determine whether the magnitude of associations between *AVPR1A* and sociability differed for males and females.

Finally, we also examined associations between *AVPR1A* and several additional measures of temperament to gauge the specificity of the hypothesized genetic effects. We reasoned that if *AVPR1A*'s influence on neurotransmission was specific to processes involved in social interaction (i.e., it is a purely "social neuropeptide"), one might expect that this gene would exhibit weak or null associations with other aspects of temperament. These tests will be discussed in greater depth in the following sections.

Method

Participants.

See Study 1 for information regarding sample ascertainment and characteristics.

Measures.

EAS Temperament Survey.

The EAS Temperament Survey for Children (Buss & Plomin, 1984) is a 20-item questionnaire for parents designed to elicit information on their child or adolescent's temperament. Items are subdivided into 4 scales: emotionality, activity, shyness, and sociability. Factor analysis of the structure of the survey has supported discriminant validity among these four subscales, although a three-factor solution (combining shyness and sociability scales) also appears to be acceptable as items within these scales exhibit a moderate negative correlation (Boer & Westenberg, 1994; Mathiesen & Tambs, 1999). Heritability has been shown to be moderate to large for the EAS scales (Benish-Weisman et al., 2010).

For the present study, we were primarily interested in the sociability scale, as this dimension is the most relevant for examining the hypothesized associations between neuropeptide genes and participants' social behavior. For the sociability scale, items included "Likes to be with people," "Finds people more stimulating than anything else," "Prefers playing with others rather than alone," and "Is something of a loner" (reverse coded). The sociability

scale has also shown fairly good internal consistency (Cronbach's alpha [α] = 0.60-.74) and inter-rater agreement (kappa [K] = 0.67) (Boer & Westenberg, 1994; Mathiesen & Tambs, 1999) and exhibits face validity. Sociability was modeled as a single latent factor indicated by both mother and father reports (when available) of the four items that comprise this scale.

We also conducted a set of secondary analyses to test whether the hypothesized effects of variation in *AVPR1A* were specific to "social" aspects of temperament, as one may expect if AVP is a purely "social" neuropeptide, or whether nonspecific effects could be found for *AVPR1A* on other aspects of temperament. In this secondary set of analyses, we examined associations between AVPR1A and the three remaining EAS scales: shyness, activity, and emotionality. Like the sociability scale of the EAS, the shyness scale is comprised of items related to social behavior (i.e., "Takes a long time to warm up to strangers," "Makes friends easily" [reverse coded], "Tends to be shy," "Is very sociable" [reverse coded], "Is very friendly with strangers" [reverse coded]), although the extent to which shyness and sociability scale items are related appears to depend on age. In younger children (< 8.3 years), these scales have shown moderate to high correlations, whereas in older children (>8.3 years) these scales have shown only small to moderate correlations (Boer & Westenberg, 1994). One possible explanation for this is that a greater number of factors, such as adjustment difficulties, anxiety, and depression, contribute to shyness as children progress in age (Karevold, Ystrom, Coplan, Sanson, & Mathiesen, 2012; Wang, Rubin, Laursen, Booth-Laforce, & Rose-Krasnor, 2013). Internal consistency (α = .81-.88) and inter-rater reliability (K = .74) for the shyness scale are high. Given our sample's age range and the partial overlap between these dimensions, one might expect that if *AVPR1A* significantly influences sociability, it may also influence shyness to some extent.

Two other aspects of temperament derived from EAS scales, activity and emotionality, were examined in these secondary analyses. The activity scale includes items "Is very energetic,"

"Prefers quiet, inactive games to more active ones" (reverse coded), "Is always on the go," "When he moves about, he usually moves slowly" (reverse coded), and "Is off and running as soon as he wakes up in the morning." The emotionality scale includes items "Cries easily," "Reacts intensely when upset," "Tends to be somewhat emotional," "Often fusses and cries," and "Gets upset easily." Acceptable to good internal consistency and inter-rater reliability have also been demonstrated for these scales (α = .71-.82 and K = .72 for activity; α = .78-.80 and K = .58 for emotionality). Interestingly, items on the sociability scale have exhibited moderate correlations with items on the activity scale in older children (Boer & Westenberg, 1994), suggesting partial overlap in these factor dimensions, but there appears to be little or no association between sociability and items on the emotionality scale at any age. Thus, given its hypothesized role in social behavior, one might expect *AVPR1A* to have a minor influence on activity scores but not on emotionality scores.

Genotyping & Imputation.

See Study 1 for information regarding DNA collection, genotyping, and imputation procedures in this sample.

Analyses

Modeling genotype-phenotype associations.

Sociability.

We used Mplus statistical software version 7.0 (Muthén & Muthén, 1998-2012) to conduct gene-based tests of the hypothesized associations between *AVPR1A* and sociability. First, we used confirmatory factor analysis (CFA) with a robust weighted least squares estimator (WLSMV) to model sociability as a continuously-distributed latent factor indicated by the mother- and father-reported EAS sociability items (4 items per parent). The fit of this measurement model and the significance of the factor loadings were assessed prior to the

factor's inclusion in the overall structural model. As in the previous study, we selected a small set of covariates for inclusion in this model (age, age^2, sex, proportion European ancestry) after observing strong multicollinearity among a larger set of covariates that were initially included in the model (age, age^2, sex, age X sex, age^2 X sex, proportion European ancestry, proportion African ancestry, and proportion Hispanic ancestry). Once again, we modeled *AVPR1A* as a continuous latent genetic factor with the eight genotyped SNPs as categorical factor indicators. See the previous study for information regarding the fit of this measurement model. Goodness of fit in all models was characterized by the following indices (also previously discussed): AIC, BIC, RMSEA, CFI, TLI, SRMR.

In the structural model, the latent sociability factor was regressed on the covariates and *AVPR1A*. Refer to Figures 7-11 for a visualization of these models. We used a *z*-test to assess the significance of the regression coefficient for the regression of the latent sociability factor on *AVPR1A*. In addition, the R^2 contribution of the *AVPR1A* genetic factor to the latent sociability factor was computed as the difference in R^2 for the full structural model and that of the model containing only the latent sociability factor and its covariates. Based on our findings for genotyping quality and power in the previous study, we tested the structural models in the same four sample types: 1) the full sample, 2) the high-quality subsample, 3) the imputed sample, and 4) the imputed, high-quality subsample.

Sex differences in *AVPR1A*-sociability associations.

We also tested whether sex differences were present in the magnitude of associations between *AVPR1A* and sociability by including an interaction term (*AVPR1A* X sex) in the final, best-fitting structural model. The significance of the interaction term was determined using a z-test of its regression coefficient (i.e., the unidirectional path from the latent *AVPR1A* X sex factor to the latent sociability factor) relative to its standard error.

Shyness, activity, and emotionality.

We tested for the specificity of *AVPR1A*'s role in social behavior by replicating the previously described structural model with the three remaining temperament scales of the EAS: shyness, activity, and emotionality. Each structural model included an individual latent temperament factor (i.e., shyness, activity, or emotionality), which was indicated by the appropriate mother- and father-reported EAS items regressed on the aforementioned covariates, and the latent *AVPR1A* gene factor. We used *z*-tests to assess the significance of all regression coefficients and computed the R^2 for each model in the previously described manner. Because these analyses were a secondary focus and we wished to limit the overall number of statistical tests that were conducted in the present study, we chose to analyze associations between *AVPR1A* and these three additional temperament dimensions exclusively within the full sample.

Results

Genotyping Quality

See Tables 1-6 of Study 1 for information regarding sample/subsample attributes and the results of the quality control analyses for *AVPR1A*.

Modeling *AVPR1A* and Sociability

Raw data.

Full sample.

See Table 1 for results. In the measurement model of sociability, all mother- and father-reported items loaded significantly on the latent sociability factor, and fit statistics indicated fair to good model fit. Of the covariates included in the model, only sex was significantly associated with sociability. As noted previously, the fit statistics for the measurement model of *AVPR1A* indicated fairly poor model fit (i.e., there was excess variation in the SNPs that was not

adequately "captured" by the latent *AVPR1A* factor). Thus, for the subsequent structural models, we once again chose to include all 8 SNPs in the initial test of association and then drop each of the 2 highly correlated SNPs in subsequent nested models.

The full structural model, which included the latent sociability factor with 8 indicators, the reduced number of covariates, and the latent *AVPR1A* genetic factor with 8 SNP indicators, exhibited acceptable fit according to the model fit statistics. The coefficient for the regression of aggression on *AVPR1A* revealed a significant association between the latent *AVPR1A* factor and sociability, B = -.083, SE = .042, z = -1.98, p = .047, R^2 = .011. As previously demonstrated in Study 1, removing rs1174808 as an indicator of *AVPR1A* improved model fit, but this also reduced the regression coefficient and increased the standard error of the association, rendering it nonsignificant, B = -.074, SE = .052, z = -1.44, p = .149, R^2 = .009 (although the change in R^2 from the previous model was modest). Additionally, removing rs1587097 as an indicator of *AVPR1A* also had negative impact on the regression coefficient but did not further affect its standard error, resulting in a nonsignificant association between *AVPR1A* and aggression, B = -.061, SE = .052, z = -1.159, p = .246, R^2 = .006.

We also tested whether associations between *AVPR1A* and sociability differed for males and females by including an *AVPR1A* X sex interaction term in the final model. The interaction term did not emerge as significant, providing no evidence that these associations differed for males and females z = -1.11, p = .267.

High-quality subsample.

Removing poorly genotyped individuals from the sample once again resulted in a loss of data for 86 participants for these analyses, or ~15% of the sample. As in the full sample, all 8 items loaded on sociability in the structural models for the high-quality subsample, and model fit was acceptable to good across models. Model fit was improved by dropping each of the 2

highly correlated SNPs from the model. For the 8 SNP model, the regression coefficient and standard error were more similar to those seen in the 7 SNP model in the full sample, yielding a nonsignificant association, B = -.073, SE = .054, z = -1.35, p = .176, R^2 = .008. For the 7 and 6 SNP models in the high-quality subsample, the pattern of regression coefficients and standard errors for the association between *AVPR1A* and sociability were very similar to those seen in the equivalent models within the full sample, yielding nonsignificant associations, B = -.074, SE = .053, z = -1.37, p = .169, R^2 = .008 for 7 SNPs, B = -.061, SE = .054, z = -1.13, p = .259, R^2 = .006 for 6 SNPs.

Once again, the *AVPR1A* X sex interaction term did not emerge as significant, providing no evidence that these associations differed by sex, z = -1.03, p = .303.

Imputed sample.

Fit statistics for the models in the full imputed sample were once again comparable but slightly worse than the fit statistics for the models in the sample that used only raw data, an observation that may have resulted from the increase in observations across SNPs. All imputed SNPs loaded significantly on the latent gene factor for *AVPR1A*. The structural models exhibited acceptable but not good fit to the data, and the fit of the models was improved somewhat by removing the two highly correlated SNPs from the model. The regression coefficients and standard errors for these models were very similar to those seen in the raw data. Associations between *AVPR1A* and sociability were marginally significant in the 8 SNP model, B = -.082, SE = .046, z = -1.79, p = .073, R^2 = .011, and nonsignificant in the subsequent models B = -.081, SE = .050, z = -1.61, p = .108, R^2 = .011 for 7 SNPs, B = -.066, SE = .049, z = -1.34, p = .179, R^2 = .007 for 6 SNPs.

Again, the *AVPR1A* X sex interaction term introduced in the final model did not emerge as significant, providing no evidence for sex differences in associations between males and females, z = -1.22, p = .224.

Imputed high-quality subsample.

Fit statistics for the models utilizing the imputed high-quality subsample were acceptable to good, and dropping the two highly correlated SNPs from the model resulted in small improvements in model fit. As previously observed in the high-quality subsample using the raw data, removing individuals with poor genotyping quality from the sample resulted in reductions in the regression coefficients and increases in the standard errors across models, yielding nonsignificant associations, B = -.074, SE = .054, z = -1.38, p = .167, R^2 = .009 for 8 SNPs, B = -.071, SE = .054, z = -1.31, p = .190, R^2 = .008 for 7 SNPs, B = -.058, SE = .055, z = -1.05, p = .293, R^2 = .005 for 6 SNPs.

Once again, no significant sex differences were found in the associations between *AVPR1A* and sociability, as the *AVPR1A* X sex interaction term did not emerge as significant z = 1.105, p = .310.

Testing individual SNPs.

As in the previous investigation, we chose to complement the gene-based analyses of the hypothesized association between *AVPR1A* and sociability with a more traditional SNP-based approach. This additional analysis allowed us to test whether any of the individual SNPs within *AVPR1A* contributed unique variance in sociability over and above that contributed by common variation among the SNPs. The latent sociability factor was regressed on the aforementioned covariates as well as the 8 genotyped SNPs (coded as binary categorical variables in the manner previously described) within a single model. We also applied the

Bonferonni correction to compensate for multiple testing (i.e., for eight significance tests) in this model, resulting in a SNP-based critical $p = .00625$.

See Table 2 for results of the SNP-based tests. As noted previously, fit statistics and parameter estimates from the SNP-based tests were identical for the full and high-quality samples in the raw data as individuals missing information on any of the predictor variables were automatically excluded from the analysis. In the raw and imputed full samples, no SNPs emerged as significant predictors of sociability before or after correction for multiple testing. In the imputed high-quality subsample, 1 SNP (rs11174808) emerged as a significant predictor of sociability and remained significant following the Bonferonni correction for multiple testing, $z = 6.58$, $p < .001$.

Modeling *AVPR1A* and Shyness, Activity, and Emotionality

See Table 3 for information regarding model fit and significance of the structural models examining the shyness, activity, and emotionality dimensions regressed on the latent *AVPR1A* gene factor. The 8 SNP models exhibited acceptable fit, and the fit of each model was improved by removing the 2 highly correlated SNPs as previously described.

For the models that included the shyness dimension, none of the covariates emerged as significant, and one SNP (rs4763062) did not significantly load on the *AVPR1A* factor ($p = .072$). In the 8 SNP model, AVPR1A emerged as a significant predictor of shyness, $B = .094$, $SE = 0.044$, $z = 2.13$, $p = .033$, $R^2 = .015$. The removal of one or both highly correlated SNPs from this model had a large, negative impact on the magnitude of the regression coefficient but did not impact the standard error, yielding nonsignificant associations for the reduced models, $B = .053$, $SE = 0.045$, $z = 1.19$, $p = .232$, $R^2 = .005$ for 7 SNPs, and $B = .052$, $SE = 0.044$, $z = 1.17$, $p = .240$, $R^2 = .004$ for 6 SNPs.

For the models that included activity, only age emerged as a significant covariate. One SNP (rs4763062) also did not load significantly on the latent gene factor. The magnitude of the regression coefficient was very large in the 8 SNP model, resulting in a significant association between *AVPR1A* and activity B = -.157, SE = 0.049, z = -3.20, p = .001, R^2 = .033, but the removal of one or both highly correlated SNPs from the model substantially reduced the magnitude of this coefficient to the point of nonsignificance, B = -.062, SE = 0.055, z = -1.13, p = .260, R^2 = .005 for 7 SNPs, B = .-040, SE = 0.056, z = -.72, p = .473, R^2 = .002 for 6 SNPs.

For the models that included emotionality, none of the covariates emerged as significant, and one SNP (rs11174803) did not significantly load on *AVPR1A*. Surprisingly, *AVPR1A* emerged as a significant predictor of the latent emotionality dimension in the 8 SNP model, with a comparatively large effect size, B = .156, SE = 0.035, z = 4.43, p = .001, R^2 = .042. Removing the highly correlated SNPs from this model substantially reduced the regression coefficient and increased the standard error, resulting in nonsignificant associations in the reduced models, B = -.024, SE = 0.056, z = -.44, p = .661, R^2 = .001 for 7 SNPs, and B = .007, SE = 0.057, z = .12, p = .903, R^2 = .000 for 6 SNPs.

Discussion

The primary goal of this study was to explore associations between *AVPR1A* and an index of prosocial behavior, sociability, using a novel approach that modeled the gene as a latent factor indicated by genotyped SNPs. Previous findings suggested that *AVPR1A* may play a role in altruism (Avinun et al., 2011; Knafo et al., 2008) and romantic relationships (Walum et al., 2008), but the genetic markers implicated in these associations and the direction of allelic effects have been inconsistent between studies. We aimed to reduce this inconsistency in our own investigation and future investigations of this gene by introducing a new method of

examining genetic association, the benefits of which included more comprehensive coverage of the gene through the exploitation of LD and increased power to detect statistical associations.

Prior to examining the effects of *AVPR1A* on sociability in our own sample, we hypothesized that variation across the gene would be associated with variation in the phenotype. Our findings however, did not appear to support this hypothesis. Although the 8 SNP models of *AVPR1A* were significantly associated with sociability in the full sample using raw data and marginally associated with sociability in the full sample using imputed data, these associations with sociability were not significant in either of the high-quality samples, and neither the 7 or 6 SNP models were associated with sociability in any of these samples. Based on these findings, it appears that common variation in *AVPR1A* may not play a role in children's sociability, at least in this sample. We also tested whether the magnitude of associations between *AVPR1A* and sociability differed for boys and girls in the sample and the interaction term was also nonsignificant, suggesting that there were no sex differences present.

As in Study 1, we conducted a post-hoc set of analyses in order to determine whether there were observable differences in the regression coefficients between boys and girls that were not detected by the *AVPR1A* x sex interaction test. Once again, the complexity of these models required that we remove ancestry as a covariate and collapse across the heterozygous and homozygous categories for the minor alleles of rs11174803 and rs11174820 in order to run the models separately for boys and girls. The results of the post-hoc analyses were similar to those found previously in Study 1 in the context of aggression; girls' sociability was significantly associated with variation in the *AVPR1A* factor, $B = -.273$, $SE = .067$, $z = -4.06$, $p < .001$, $R^2 = .151$, whereas boys' sociability was not $B = -.036$, $SE = .060$, $z = -.59$, $p = .555$, $R^2 = .003$. These findings indicate that *AVPR1A* may indeed affect prosocial behavior in a sexually dimorphic manner, although the large magnitude suggested by the R^2 effect size in females suggest that they may

be the result of problems inherent in the model given the size of the divided sample (e.g., overfitting). Nonetheless, it is important that future investigations test for *AVPR1A*'s effects on sociability using larger individual samples of males and females, which will allow investigators to conduct these analyses stratified by sex while controlling for participant ancestry. This will also help to ensure that any significant associations are not confounded by the effects of population stratification.

As in the previous study, we conducted complementary analyses using a more traditional SNP-based approach in order to determine whether any of the individual genotyped SNPs contributed unique variance to the phenotype of interest. In the samples that utilized raw data and in the high-quality imputed data sample we found one SNP, rs11174808, that was significantly associated with sociability after adjusting for multiple testing. Nonetheless, upon further examination the minor allele frequency for this marker was .01 in the raw data sample and .005 in the imputed data sample, indicating that variation in this SNP is fairly rare. It is thus possible that the observed association between rs11174808 and sociability scores is a statistical artifact due to a few anomalous cases. Incidentally, rs11174808 was the same SNP that was dropped from the 8 SNP models to produce the 7 SNP models in the gene-based tests of *AVPR1A*. This may explain why some of the findings for these tests across different models and samples were inconsistent.

A secondary goal of this study was to explore whether *AVPR1A*'s effects were exclusive to social behavior. We examined associations between *AVPR1A* and three additional dimensions of temperament—shyness, activity, and emotionality—taken from the same temperament measure as sociability. Because both shyness and activity had previously demonstrated small to moderate correlations with sociability but emotionality appears unrelated (Boer & Westenberg, 1994), we hypothesized that *AVPR1A* might exhibit small associations with the shyness and

activity temperament dimensions but would not be associated with emotionality. Surprisingly, we found that *AVPR1A* was significantly associated with all three of these temperament dimensions in the 8 SNP models, and the magnitude of associations for these dimensions was similar to that observed for sociability. Nonetheless, *AVPR1A* was not associated with any dimension in the 7 SNP or 6 SNP models, suggesting these associations were highly dependent on the presence of the very rare rs11174808 variant, and as such, it is likely that one or two anomalous scores were responsible for these associations. Overall, *AVPR1A* did not show consistent evidence for association with any measured aspect of child temperament, including sociability, once that SNP was excluded.

There were several limitations to the present study. Although we did not find compelling evidence for an association between *AVPR1A* and prosocial behavior, it is important to note that we only examined one construct related to prosocial behavior, sociability. Sociability is conceptualized as one's interest and enjoyment in spending time with others. We selected this temperament dimension as an outcome measure because it is germane to the study of social relationship formation and had shown previous evidence for temporal stability as well as heritability in children. Nonetheless, prosocial behavior is conceptualized as behavior intended for the benefit of others and/or the promotion of social bonding. Although sociability as a construct certainly may reflect one's motivation to participate in prosocial behavior, because it does not provide an indication of the frequency of duration of such behaviors these two constructs may only partially overlap. Future studies should apply the gene-based test introduced in the present investigation to the examination of other prosocial phenotypes, including altruism and nurturance, using instruments designed to gauge the frequency of prosocial actions in much the same way that previous studies have done in the nonhuman animal literature (e.g., rats' time spent in contact with pups; Bosch & Neumann, 2008). By

conducting additional studies using a range of specific prosocial phenotypes, we will be able to conclude with greater confidence whether *AVPR1A* influences variation in this construct.

As in the previous study, we were also limited by our sample size. Phenotypic information was only available for approximately 70% of genotyped children, resulting in a considerable reduction in our potential sample size and statistical power. The reduction in sample size may have impacted our findings, as most of the SNPs included in our models exhibited low minor allele frequencies (< .05) in the present study, including the aforementioned rs11174808 variant. Because this small sample size, in conjunction with the low minor allele frequency of rs11174808, resulted in very few observations for individuals who were homozygous for the minor allele, we may have been more susceptible to anomalous associations in the present study (see earlier Discussion) than we would have been with a larger sample. Consequently, it will be important for future studies to employ larger samples and to rely on collaborative multi-sample designs.

Finally, it is important to note that our sample included both children and adolescents across a fairly wide age range, and this inclusiveness may have impacted our results. There is evidence that the heritability of prosocial behavior may change throughout childhood, as one study reported an increase in the heritability of these behaviors from 32% at age 2 to 61% at age 7 (Knafo & Plomin, 2006). Although this change in the relative contribution of heritable influences to the variance in prosociality does not necessarily reflect a change in the genes implicated in these behaviors or the magnitude of their effects, it demonstrates that we cannot assume that the measurement of this construct, its variability in the general population, or the influences underlying its variability are constant. Although our sample size was not large enough to permit stratified analysis in the current investigation, future studies may explore whether

AVPR1A's effects differ across age by examining these effects separately in child and adolescent samples or by using age as a continuous moderator.

The current investigation was the first to utilize a latent gene-based test of association to examine whether AVP-ergic system genes play a role in prosocial behavior. We found that common variation in this gene was not robustly associated with variation in sociability for boys or girls or with variation in any other measured aspects of child temperament. Future studies should investigate whether *AVPR1A* is associated with other forms of prosocial behavior, including the frequency and duration of prosocial acts such as altruism or nurturance, at different time points throughout childhood and adolescence.

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Table 1

Model results for gene-based tests of AVPR1A and sociability

	χ^2	*p*	N	RMSEA	CFI	TLI	WRMR	AIC	BIC	*Coefficient*	*SE*	*Z* (reg.)	*p*
MEASUREMENT MODELS[1]													
SOC only	106.21	<.01	473	.095	.95	.93	1.08	186.21	352.57				
SOC with all covariates[2]	93.66	.08	434	.023	.98	.98	.81	189.66	385.17				
SOC with reduced covariates	114.70	<.01	434	.057	.96	.95	.98	202.70	381.91				
STRUCTURAL MODELS													
Raw Data													
Full sample													
SOC on AVPR1A (8 SNPs)	548.16	<.01	573	.064	.83	.81	1.73	686.16	986.61	-0.083	0.042	-1.98	.05*
SOC on AVPR1A (7 SNPs)	341.76	<.01	573	.049	.91	.89	1.36	473.76	761.15	-0.074	0.052	-1.44	.15
SOC on AVPR1A (6 SNPs)	252.96	<.01	573	.041	.94	.93	1.18	378.96	653.29	-0.061	0.052	-1.16	.25
High-quality subsample													
SOC on AVPR1A (8 SNPs)	509.61	<.01	489	.066	.82	.80	1.67	643.61	924.49	-0.073	0.054	-1.35	.18
SOC on AVPR1A (7 SNPs)	330.81	<.01	489	.051	.90	.89	1.34	460.81	733.31	-0.074	0.053	-1.37	.17

SOC on AVPR1A (6 SNPs)	243.74	<.01	489	.043	.94	.93	1.16	367.74	627.67	-0.061	0.054	-1.13	.26
Imputed Data													
Full sample													
SOC on AVPR1A (8 SNPs)	599.54	<.01	573	.068	.80	.77	1.84	737.54	1037.75	-0.082	0.046	-1.79	.07[t]
SOC on AVPR1A (7 SNPs)	399.57	<.01	573	.055	.87	.85	1.51	531.57	818.73	-0.081	0.050	-1.61	.11
SOC on AVPR1A (6 SNPs)	312.66	<.01	573	.050	.91	.89	1.36	438.66	712.77	-0.066	0.049	-1.34	.18
High-quality subsample													
SOC on AVPR1A (8 SNPs)	496.95	<.01	489	.065	.82	.80	1.65	630.95	911.70	-0.074	0.054	-1.38	.17
SOC on AVPR1A (7 SNPs)	334.33	<.01	489	.052	.89	.88	1.36	464.33	736.70	-0.071	0.054	-1.31	.19
SOC on AVPR1A (6 SNPs)	247.13	<.01	489	.044	.93	.92	1.17	371.13	630.93	-0.058	0.055	-1.05	.29

Note: [1]See Table 7 in Study 1 for measurement model of AVPR1A; [2]Despite favorable fit statistics, covariances within the measurement model of sociability that included all covariates indicated strong multicollinearity among predictors, so a reduced number of covariates was selected for inclusion in the following structural models; SOC indicates the latent sociability factor; *indicates significance of the regression coefficient at .05 level; [t]indicates marginal significance at .10 level

Table 2

Model results for SNP-based tests of AVPR1A and sociability

	χ^2	*p*	N	RMSEA	CFI	TLI	WRMR	AIC	BIC	*Coefficient*	*SE*	*Z* (regression)	*p*
Raw Data													
Full sample/High-quality subsample[a]	472.22	<.01	297	.109	.72	.66	1.85	576.22	768.29				
rs11174803										-.352	.185	-1.90	.06
rs962862										.079	.242	.33	.74
rs1587097										.097	.203	.48	.63
rs2738250										-.224	.191	-1.17	.24
rs4763062										.019	.135	.14	.89
rs11174808										2.114	.142	14.91	<.001*
rs11174820										.003	.242	.01	.99
rs7307997										.100	.120	.83	.41
Imputed Data													
Full sample	149.54	<.01	432	.032	.97	.96	.88	253.54	465.09				
rs11174803										.020	.210	.10	.92

rs962862										-.056	.175	-.32	.75
rs1587097										-.320	.233	-1.38	.17
rs2738250										-.171	.120	-1.42	.16
rs4763062										-.076	.104	-.73	.45
rs11174808										.235	1.505	.16	.88
rs11174820										-.052	.240	-.22	.83
rs7307997										.119	.107	1.11	.27
High-quality subsample	282.03	<.01	374	.068	.90	.88	1.35	386.03	590.09				
rs11174803										.008	.190	.04	.97
rs962862										-.085	.183	-.46	.64
rs1587097										-.317	.211	-1.50	.13
rs2738250										-.167	.148	-1.13	.26
rs4763062										-.002	.118	-.02	.98
rs11174808										6.086	.925	6.58	<.001*
rs11174820										-.066	.225	-.29	.77
rs7307997										.096	.104	.93	.35

Note: covariates included in regression analysis include proportion European ancestry, sex, age, age^2; * indicates significance at p = .00625 level; [a]because models were estimated based on individuals who had information available for all predictor variables, fit statistics and parameter estimates from the SNP-based tests were identical for the full sample and high-quality subsample within the raw dataset.

Table 3

Model results for gene-based tests of AVPR1A and shyness, activity, and emotionality

	χ^2	*p*	N	RMSEA	CFI	TLI	WRMR	AIC	BIC	*Coefficient*	*SE*	*Z* (reg.)	*p*
Shyness													
SHY on AVPR1A (8 SNPs)	747.91	<.01	573	0.069	0.83	0.81	1.91	905.91	1249.91	.094	.044	2.13	0.03*
SHY on AVPR1A (7 SNPs)	616.68	<.01	573	0.064	0.86	0.85	1.76	768.68	1099.62	.053	.045	1.19	0.23
SHY on AVPR1A (6 SNPs)	548.50	<.01	573	0.064	0.88	0.87	1.69	694.50	1012.36	.052	.044	1.17	0.24
Activity													
ACT on AVPR1A (8 SNPs)	662.59	<.01	573	0.063	0.82	0.80	1.76	820.59	1164.59	-.157	.049	-3.20	0.001*
ACT on AVPR1A (7 SNPs)	480.85	<.01	573	0.053	0.88	0.86	1.51	632.85	963.78	-.062	.055	-1.13	0.26
ACT on AVPR1A (6 SNPs)	396.25	<.01	573	0.050	0.90	0.89	1.40	542.25	860.12	-.040	.056	-0.72	0.47
Emotionality													
EMO on AVPR1A (8 SNPs)	911.31	<.01	573	0.069	0.85	0.83	1.98	1089.31	1476.85	.156	.035	4.43	<.001*
EMO on AVPR1A (7 SNPs)	547.41	<.01	573	0.050	0.92	0.91	1.51	719.41	1093.88	-.024	.056	-0.44	0.66
EMO on AVPR1A (6 SNPs)	463.52	<.01	573	0.047	0.94	0.93	1.40	629.52	990.94	.007	.057	0.12	0.90

Note: SHY indicates the latent factor dimension for shyness; ACT indicates the latent factor dimension for activity; EMO indicates the latent factor dimension for emotionality; *indicates significance of the regression coefficient at $p < .05$; all reported results are for the full sample using raw data.

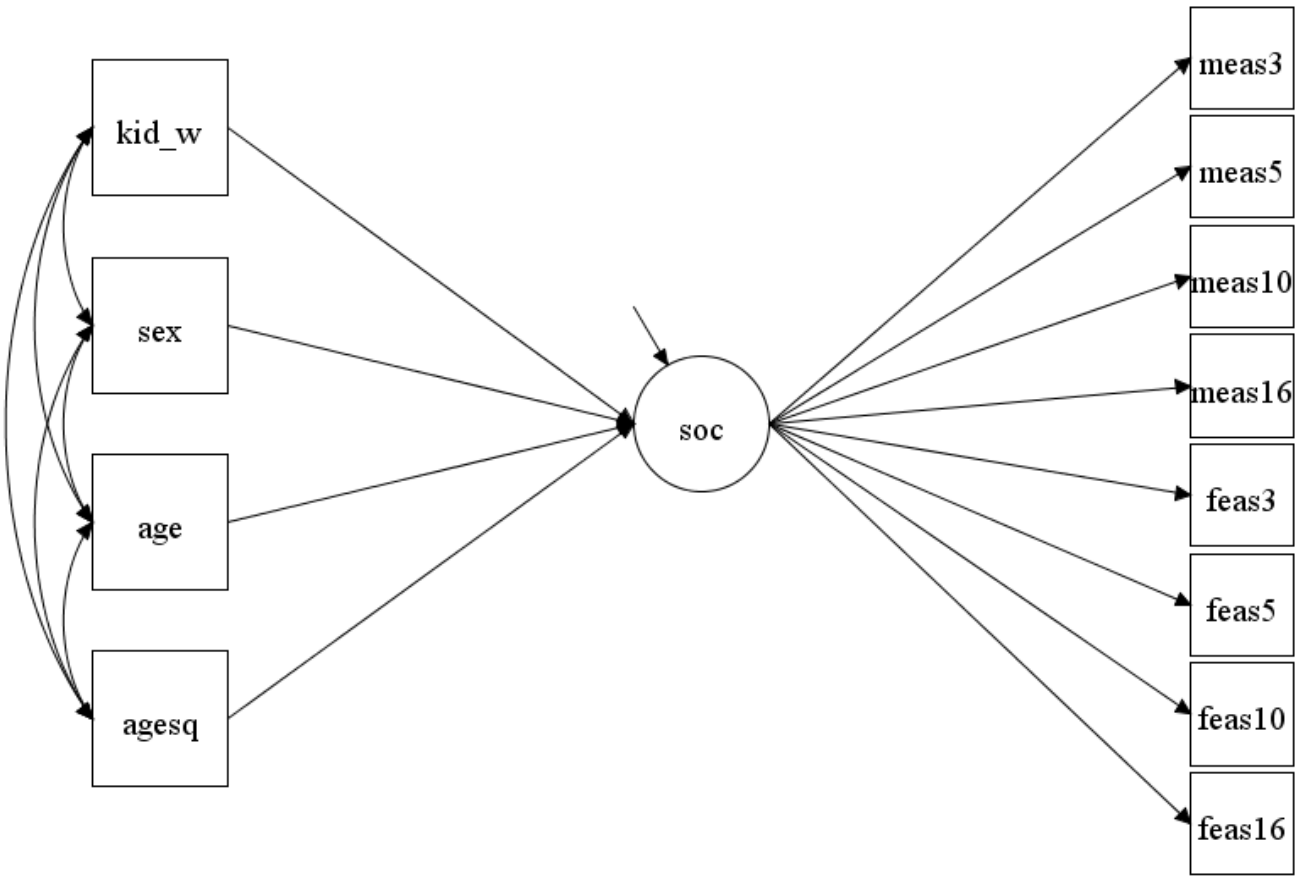

Figure 7. Latent sociability factor with reduced covariates. Covariates are on the left side of the latent sociability factor; factor indicators (EAS items, mother-reported meas and father-reported feas) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

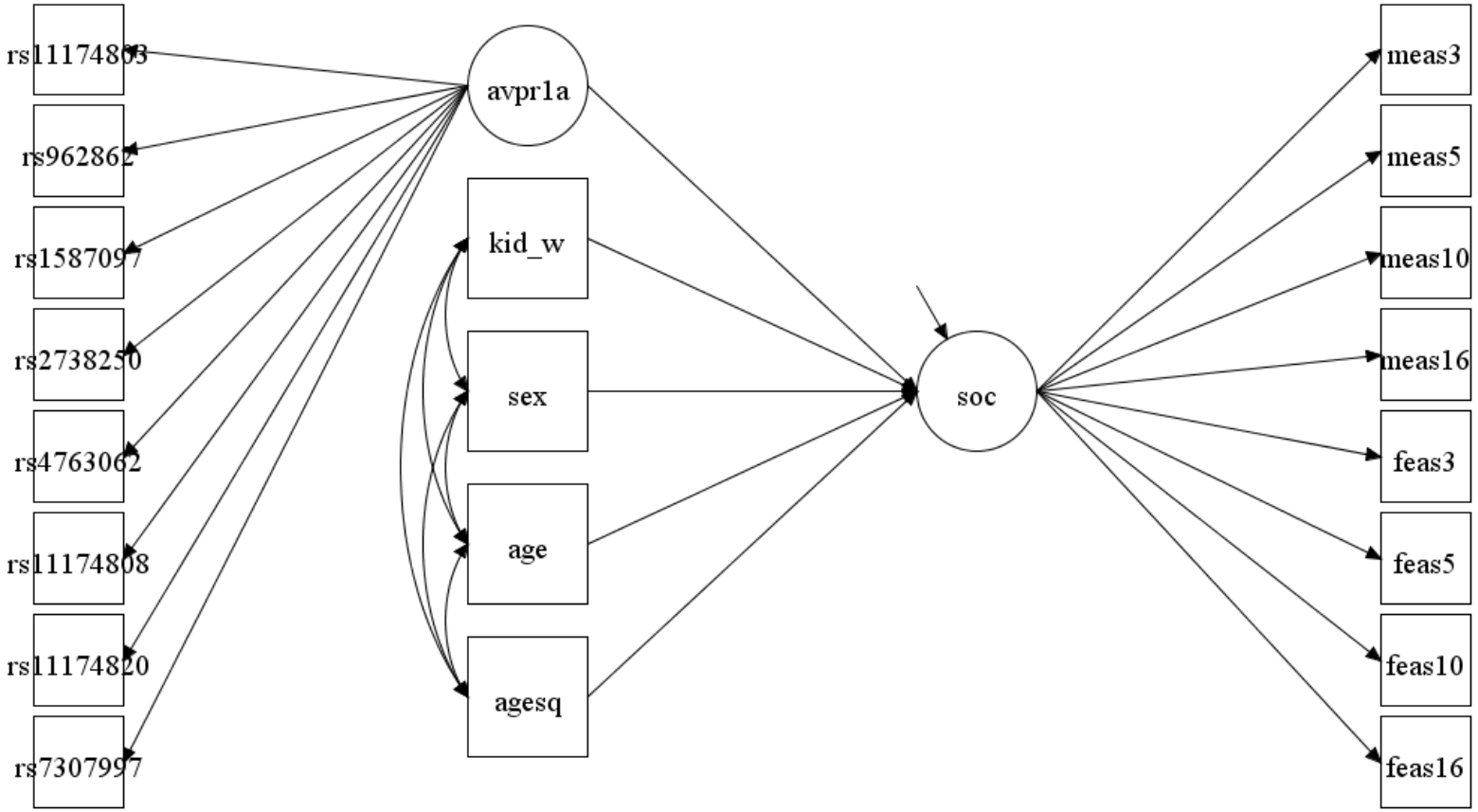

Figure 8. Latent sociability factor with reduced covariates and *AVPR1A* (8 SNPs). Covariates are on the left side of the latent sociability factor; factor indicators (EAS items, mother-reported meas and father-reported feas) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

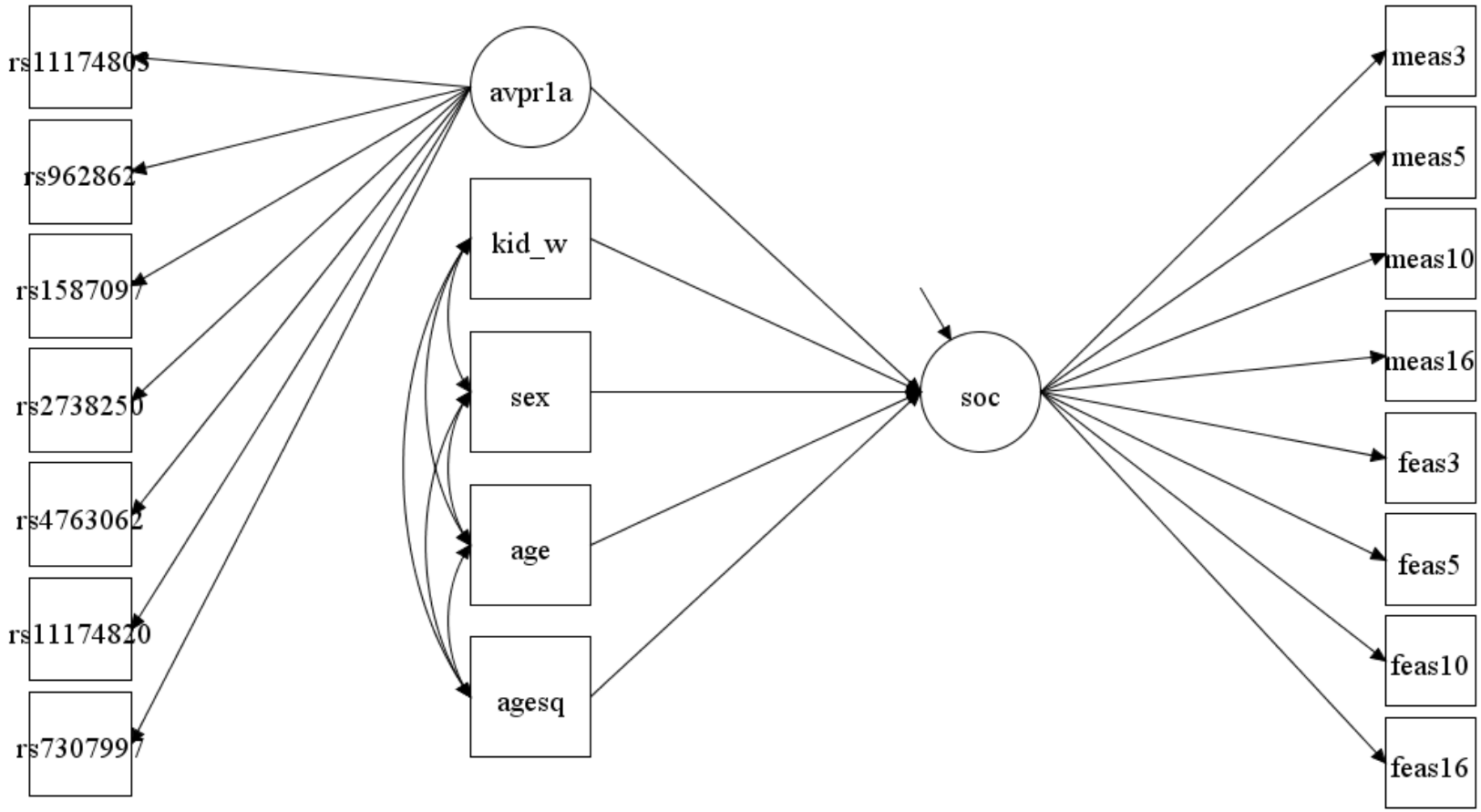

Figure 9. Latent sociability factor with reduced covariates and *AVPR1A* (7 SNPs). Covariates are on the left side of the latent sociability factor; factor indicators (EAS items, mother-reported meas and father-reported feas) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

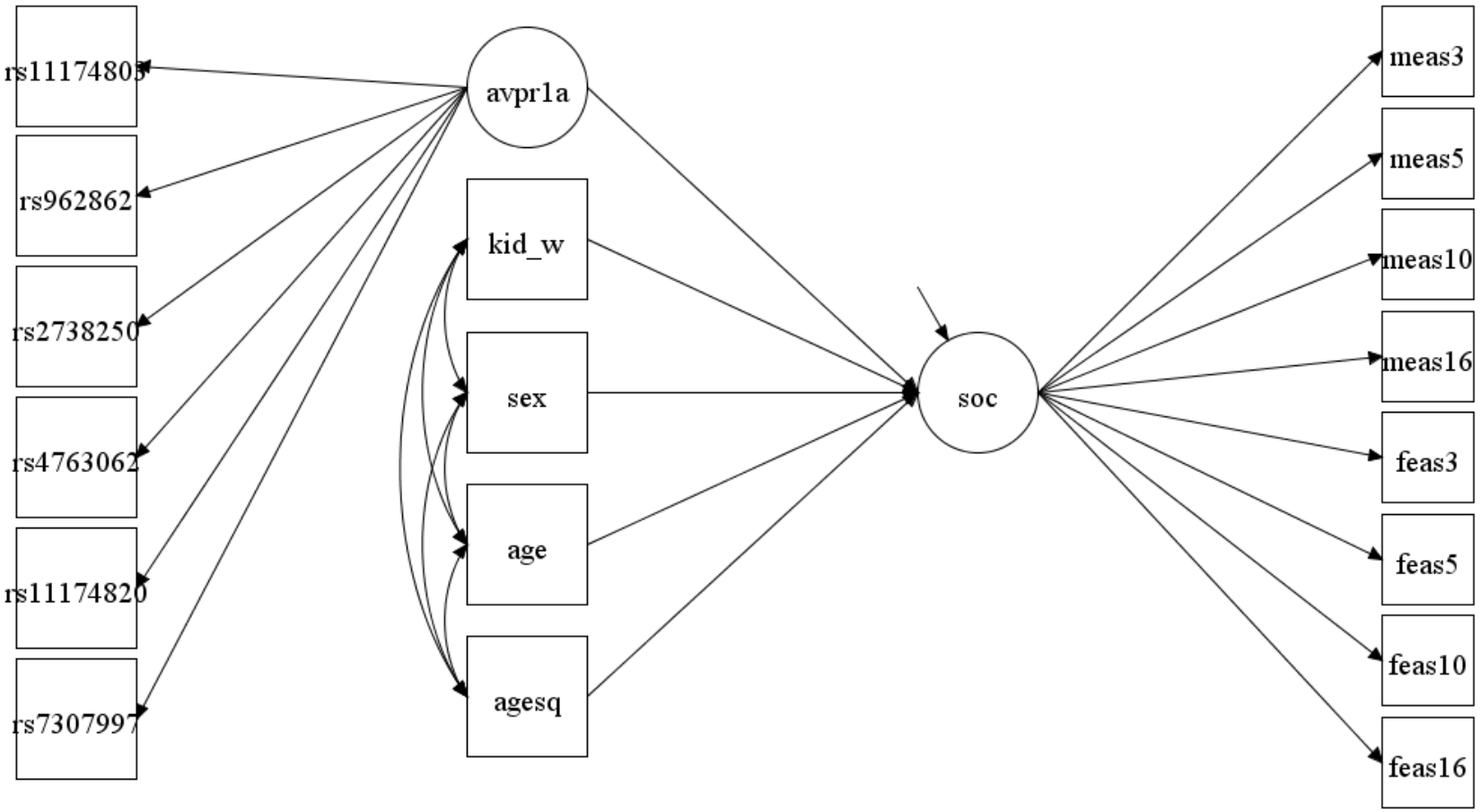

Figure 10. Latent sociability factor with reduced covariates and *AVPR1A* (6 SNPs). Covariates are on the left side of the latent sociability factor; factor indicators (EAS items, mother-reported meas and father-reported feas) are on the right side. From top to bottom, covariates European ancestry, sex, age, age^2.

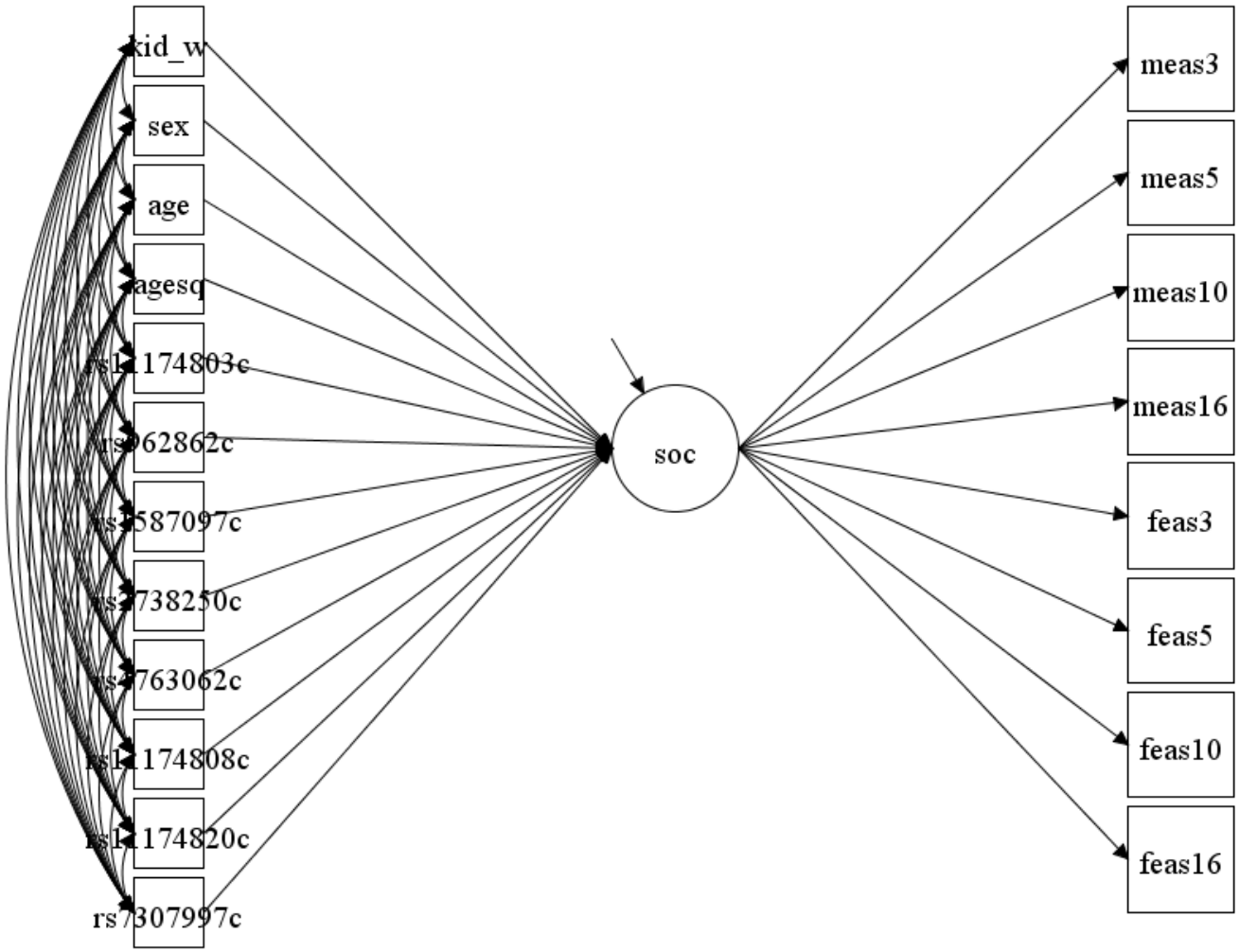

Figure 11. SNPs-only test of latent sociability factor and *AVPR1A*. SNPs and covariates are on the left side of the latent sociability factor; factor indicators (EAS items, mother-reported meas and father-reported feas) are on the right side. From the top, covariates European ancestry, sex, age, and age^2. The "c" following each SNP rs number indicates the SNP was coded as a binary categorical variable.

General Discussion

Implications for AVP in Social Behavior

In the present investigations, we examined associations between *AVPR1A*, the AVPR1a receptor gene, and two forms of social behavior—aggression and prosociality—in children. We examined these two forms of social behavior because 1) they represent dimensions of social behavior that have previously been associated with AVP-ergic system variation in nonhuman animals and humans, and 2) they are ostensibly very distinct phenotypes; aggressive behaviors in children are typically pathological and viewed as risk factors for negative outcomes (e.g., Cleverley, Szatmari, Vaillancourt, Boyle, & Lipman, 2012; Temcheff et al., 2011), whereas prosocial behaviors are considered normative and even protective against the effects of adversity (e.g.,Crick, 1996; Griese & Buhs, 2013). Although more studies to date have examined associations between *AVPR1A* and prosocial behavior, previous research (e.g., Ferris et al., 1986; Ferris & Potegal, 1988; Insel & Hulihan, 1995; Leshner & Roche, 1977; Pitkow et al., 2001; Siegfried et al., 1984; Winslow et al., 1993) did not suggest that this gene might be more strongly associated with either phenotype. We were thus surprised to find fairly consistent and robust evidence for an association between the latent *AVPR1A* genetic factor and aggression in Study 1 but only weak, inconsistent evidence for an association between *AVPR1A* and sociability in Study 2. Given findings from previous studies relating variation in *AVPR1A* to variation in a wide range of social behaviors, including social recognition, bonding, aggression, and dominance, it is unclear why *AVPR1A* genotypes was associated only with aggression in our sample.

One plausible explanation for these differential effects is that our measurement of aggression in Study 1 was more comparable to measurements of this phenotype in previous studies within the nonhuman animal literature. The Dodge and Coie (1987) aggression scale

utilized in the current investigation asks parents to rate how well statements describing various observable aggressive behaviors (e.g., "start[ing] fights with peers,") describe their child(ren). Likewise, previous studies that have reported an association between AVP-ergic variation and aggression have typically measured the frequency and/or duration of observable aggressive behaviors toward others (e.g., Cheng & Delville, 2009; Wersinger et al., 2007; Wersinger et al., 2002). In contrast, the EAS Temperament Survey (Buss & Plomin, 1984), used in the measurement of sociability in Study 2, asks parents to report how well various statements, such as "likes to be with people" and "prefers playing with others rather than alone," characterize their child(ren). Here, parents are primarily reporting on their child's *preferences* rather than his or her *behaviors*. Not surprisingly, there is evidence that this construct is strongly related to extraversion (Plomin, 1976), and consequently sociability may not be a satisfactory measure of the quality of an individual's social relationships. Because previous studies reporting an association between AVP and prosocial behavior have primarily used behavioral approaches to measurement, including partner preference (Aragona et al., 2003), time spent with pups (Bosch & Neumann, 2008), and cooperation in the context of a social game (Rilling et al., 2013; Rilling et al., 2012), it may be that our failure to detect an association between *AVPR1A* and our measure of prosocial behavior in Study 2 was a result of differences in phenotypic measurement, rather than a true absence of association with prosocial behavior. It is easy to assume that one's preference for spending time with others should coincide with the number of relationships one has and the extent to which one engages in prosocial and relationship-building behaviors (and indeed this appears to be the case to some extent; see Boulton, 1999; McCroskey & Sheahan, 1978). Nonetheless, incomplete overlap between children's perceived preferences and their behaviors due to a host of potential reasons (e.g., reporter bias or lack of awareness, measurement error, group heterogeneity) may have resulted in a measured phenotype that

differed enough from those previously examined to diminish any association present between *AVPR1A* and prosocial behavior. In order to better understand the inconsistency in these findings, it will be important for future studies of *AVPR1A* to examine whether associations are present using more strictly behavioral measures of prosocial behavior.

In addition, our measurement of aggression in Study 1 was very broad, as we included all reactive, proactive, and nonspecific aggression items from Dodge & Coie's (1987) aggression scale. We felt that the decision to include all items was justified given the high correlations among these items as well as the high internal consistency for the overall scale. Nonetheless, it is important to note that our inclusiveness with respect to this phenotype may have resulted in the measurement of other traits outside of the bounds of aggression, such as negative emotionality. In order to test the extent to which the latent aggression factor might be associated with other aspects of child temperament, we conducted a post-hoc analysis in which we regressed this latent aggression factor on the emotionality factor previously derived from the EAS scale items (after controlling for the aforementioned covariates: ethnicity, age, sex, and age^2). The regression coefficient for this model was significant and suggested a moderate association between the two factors that contributed ~19% of the variance in aggression. In separate models, we then regressed aggression on the remaining latent temperament factors derived from the EAS scale, including sociability, shyness, and activity, to determine to whether these other dimensions of temperament might also be associated with the latent aggression factor. A small association between shyness and aggression emerged (contributing 3% of the variance in aggression), but neither sociability nor activity were significantly associated with the latent aggression factor. Thus, it appears that the broad measure of aggression may have captured some aspects of (negative) emotionality, unlike the "purer" physical aggression explicitly examined in the nonhuman animal literature. Nonetheless, based on our findings in

Study 2, it does not appear that the associations between AVPR1A and aggression were driven by its association with emotionality, as *AVPR1A* did not exhibit any consistent associations with this temperament dimension. It will be important for future research in this area to examine whether *AVPR1A*'s influence may be specific to more narrowly-defined subdimensions of aggressive behavior, or in contrast, whether these associations may be due to *AVPR1A*'s influence on broader and/or overlapping constructs such as negative emotionality or behavioral or emotional dysregulation.

Although we have heretofore referred to *AVPR1A*'s effects on childhood aggression as "consistent" and "robust," we do not mean to imply that these effects were large, which they were not. As noted in the Discussion of Study 1, these effects only contributed ~1-2% of the variance in aggression. One might infer that these findings suggest a *comparatively* large effect for *AVPR1A* given typical effect sizes for genes in both GWAS and candidate gene studies, but in absolute terms it is clear that this gene has no more than a small impact on childhood aggression overall. This finding should not be surprising; as noted in the General Introduction's discussion of "missing heritability," we should not expect common genetic variation within individual genes such as *AVPR1A* to result in large behavioral effects, because such variation resulting in significant behavioral cost (e.g., poor social skills or aggressive behavior that evokes social exclusion) would have been selected against over time (Plomin & Davis, 2009; Waddington, 1959). Even so, this presumption should not undermine the importance of identifying the specific genetic influences involved in such behaviors. In future studies, examining variation across multiple genes (particularly those relevant to AVP-ergic system functioning such as *HTR1A*), perhaps concurrently within the same structural model, may allow us to explain a greater proportion of the variance in these behaviors.

Another point worth mentioning is that the interaction tests did not provide evidence for any sex differences in the effects of *AVPR1A* on aggression or sociability. We hypothesized that these effects would differ for males and females, because sex-specific AVP-ergic effects have been previously reported in studies of social recognition and social information processing (e.g., Gabor et al., 2012; Guastella et al., 2011; Insel & Hulihan, 1995; Thompson et al., 2006), prosocial behavior (e.g., Rilling et al., 2013; Rilling et al., 2012), and aggression (e.g., Gutzler et al., 2010; Nephew & Bridges, 2008; Nephew et al., 2010). Nonetheless, such effects were not statistically significant for the phenotypes examined in our sample. Despite the nonsignificance of these interaction terms, post-hoc testing revealed observable sex differences in the regression coefficients for the genotype-phenotype associations in both studies. The regression coefficients consistently suggested that girls' (but not boys') behavior was influenced by variation in *AVPR1A*. The nature of this sex difference was surprising given previous indications that AVP is more greatly expressed (in cell number and fiber density) in typically-developing males than females across species (de Vries, 2008; Gabor et al., 2012) and the common belief that AVP has a more "essential" role in males' social information processing (Gabor et al., 2012). Further, none of the aforementioned studies examining sex differences in the effects of *AVPR1A* on social behavior reported effects exclusive to females within the sample. Clearly, more research is needed to determine whether these female-specific effects can be replicated in other samples of children and adolescents, and if so, whether hormonal changes throughout development may explain the observed inconsistencies in *AVPR1A*'s effects on male social behavior.

Indeed, as noted in Study 1, sex differences in the effects of this gene may not emerge until adolescence, which may explain why our findings differed from those reported in previous studies that utilized adult samples. This is plausible given previous evidence for the modulation

of AVP-ergic effects by testosterone in males (e.g., Bluthe et al., 1993; Bluthe et al., 1990). If these effects do not emerge until later, it would have been very difficult to detect such effects in our sample of youths aged of 6-18 years, because such effects would have been obscured by the younger individuals in the sample. As noted previously, future studies with larger sample sizes would be better able to test whether the effects of *AVPR1A* on human social behavior are sexually dimorphic and might even estimate when this dimorphism emerges by conducting stratified analyses of these effects in children, adolescents, and adults.

Implications for Gene-Based Tests with Latent Factors

The gene-based tests of association in Studies 1 and 2, which were conducted within a latent variable model framework, were largely novel. As such, we felt it necessary to conduct these analyses in a very methodical manner and provide detailed information regarding our decision-making process throughout. For instance, we chose to replicate all models using four (overlapping) sample configurations, which were described in detail in Study 1. Although some readers might have been satisfied with (and perhaps preferred) the analysis of only 1 sample configuration, as noted previously, each sample configuration presented specific gains and losses with regard to statistical power and the quality of genotypic data. By including our findings for all four sample configurations, we were able to demonstrate the robustness of this method to variations in sample attributes, including changes in sample size and genotyping accuracy, and provide examples of ways in which future investigations may handle such issues.

In addition, we found that the results of the gene-based tests were somewhat influenced by the SNPs included in the models. Although one might assume that the best practice for researchers using these methods would be to include all SNPs in the model for which genotyping is available as indicators of the latent gene factor, we found that having as many as 8 SNPs in the model (particularly when several of those SNPs were highly correlated)

sometimes caused technical issues in model-fitting and convergence. Further, the inclusion of SNPs with very low minor allele frequencies in the sample seems to have resulted in questionable results for some models (see Study 2). Based on these observations, we recommend that investigators take great care in selecting markers for inclusion in gene-based, latent variable models that are fairly common and collectively represent the majority of the variation across the gene. The impact of rare variation in the gene may be examined by oversampling for the rare allele in order to ensure adequate cell counts for quantitative analysis. Because *AVPR1A* is a fairly small gene (~6.38 kb), we were able to adequately cover the variation in this gene using only 6-8 markers. Nonetheless, many genes that may be of interest to future investigators are considerably larger (e.g., *DAT1* is ~64 kb), and it may be difficult to adequately model the variation across larger genes using a single latent variables. For larger genes, we might suggest using an exploratory factor analytic approach to identify whether the gene may be best modeled as more than one latent factor (with separate factors perhaps indicating different haplotype blocks), but at this time it is unclear what might be the biological implications of such an analysis. Clearly, more research on the applicability of this method to other genes differing in size and marker variability is needed before we can fully understand and appreciate its utility in behavior genetic research.

Questions aside, our primary goal in proposing a gene-based, latent variable approach to the analysis of genetic association was to provide a more comprehensive, parsimonious, and reliable method of determining whether variation within a gene, in this case *AVPR1A*, was associated with variation in the phenotype(s) of interest. With this goal in mind, we believe the current investigation was successful. Previous methods of testing associations with candidate genes have been viewed as unreliable because many of the findings yielded by these methods have been difficult to replicate between studies. By modeling the gene as a latent factor

indicated by variation in individual genotypes across a number of SNPs we were able to effectively capture common variation across *AVPR1A* (which was demonstrated in the finding that all 8 SNPs loaded significantly on the latent factor for the majority of these models) and determine whether this overall variation was associated with variation in each phenotype using a single statistical test. Because the inferences made from these tests regarding genetic association were on the gene-level rather than the SNP-level, they should also be more robust to between-study differences in SNP selection and sample LD (provided the coverage of variation across the gene is sufficient). There is initial evidence to indicate this may be true, as our findings for the association between *AVPR1A* and aggression were fairly robust to minor differences in sample size and marker inclusion in the current study. Nonetheless, more studies of the association between the latent *AVPR1A* factor and these social phenotypes will be needed before we can adequately support this assertion.

Implications Regarding the Role of Neuropeptide Genes in Behavior

Although more research is needed to help us understand the role of *AVPR1A* in human social behavior, there has been considerable progress in uncovering the role of the AVP-ergic system in human and nonhuman animal behavior over the past two decades. Neuroanatomical differences between similar species as well as a plethora of experimental evidence on AVP-ergic system manipulation have demonstrated that AVP receptor density and expression can impact social behaviors such as social information processing (e.g., Marshall, 2013; Thompson et al., 2006; Thompson et al., 2004), recognition (e.g., Bluthe et al., 1993; Dantzer et al., 1987; Le Moal et al., 1987), partner preference and bonding (e.g., Pitkow et al., 2001; Winslow et al., 1993), parenting (e.g., Bosch & Neumann, 2008; Bosch et al., 2010), and aggression (e.g., Delville et al., 1996a; Delville et al., 1996b; Grimes et al., 2007). Although the majority of these studies have been conducted with nonhuman species, our ability to manipulate central AVP levels through

intranasal AVP administration has recently allowed us to obtain similar experimental evidence for the role of this neuropeptide in human behavior.

As these initial findings have demonstrated temporary elevation of AVP to be associated with variation in phenotypes such as facial recognition and altruism, moving forward it will be important for us to work toward understanding the impact of more stable, long-term differences in central levels of AVP due to genetic variation. Examining stable variation in this system will allow us to better explore individual differences in socially-relevant traits such as aggression or prosociality. Further, we must do so using robust and powerful methods of examining genetic association, particularly methods such as those used in the current study that take into account both statistical power and group differences in LD, as it has been demonstrated in the recent phenomenon of "missing heritability" that a failure to do so is likely to result in inconsistencies between studies that impede further progress in the field. In this context, we must also work to understand the effects of this genetic variation on the physical properties of the AVP-ergic system itself, as it is through physical differences in the proteins expressed by these genes that genetic variation must operate to produce changes in complex behavioral phenotypes. By better understanding all stages of this process, we may be able to develop and implement targeted pharmacological interventions for individuals struggling with relevant psychological concerns such as social deficits, elevated aggression, or difficulty in social relationships.

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Appendix A

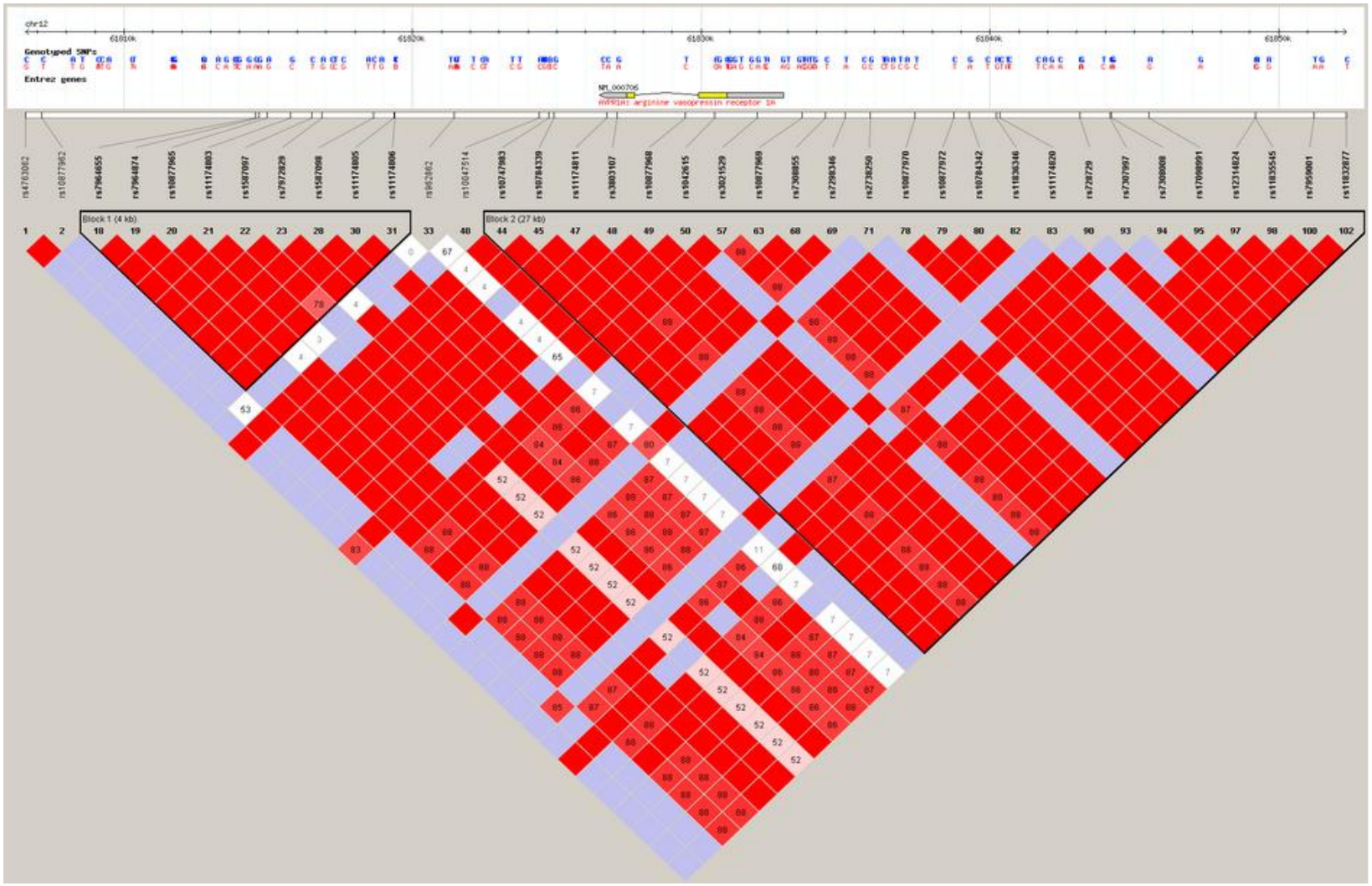
Minor Allele Frequencies of *AVPR1A* and +/- 20 kb Surrounding SNPs

SNP	location	Minor Allele Frequency
rs4763062	61806598	0.17
rs10877962	61807179	0.386
rs11174788	61809085	0.008
rs11174789	61809092	0.008
rs11174791	61809203	0
rs11174794	61809455	0.009
rs7304644	61810269	0
rs11174797	61811659	0.018
rs11174798	61811684	0.009
rs10877963	61812723	0
rs10877964	61812757	0
rs7960075	61813229	0.009
rs11174800	61813514	0
rs11830346	61813812	0
rs4763055	61813899	0
rs10784337	61813980	0.01
rs11174802	61814292	0.01
rs7964655	61814559	0.134
rs7964874	61814689	0.134
rs10877965	61814976	0.143
rs11174803	61815789	0.109
rs1587097	61816512	0.093
rs7972829	61816874	0.143
rs7976075	61817197	0
rs10877966	61817287	0
rs11174804	61817570	0.008
rs7980289	61818433	0.008
rs1587098	61818656	0.1
rs12427416	61818902	0
rs11174805	61819374	0.134
rs11174806	61819391	0.105
rs9805119	61821297	0
rs962862	61821458	0.054
rs7486346	61821482	0
rs4237924	61821538	0
rs7954346	61822074	0.009
rs7967970	61822403	0
rs7967990	61822508	0.008
rs11174808	61823679	0.009
rs10047514	61824389	0.418
rs11829406	61824540	0
rs10877967	61824542	0.008
rs11829452	61824716	0

rs10747983	61824725	0.143
rs10784339	61824913	0.142
rs11174810	61826562	0.009
rs11174811	61826743	0.134
rs3803107	61827101	0.142
rs10877968	61829452	0.143
rs1042615	61830476	0.438
rs2228153	61830593	0
rs2228154	61830868	0.018
rs3021531	61830971	0
rs3741865	61831125	0.009
rs3021530	61831356	0
rs2738255	61831708	0
rs3021529	61831947	0.134
rs11174814	61832195	0
rs3021528	61832253	0.009
rs3021527	61832794	0.009
rs2738253	61832976	0
rs11174815	61833363	0.026
rs10877969	61833506	0.107
rs3759292	61833580	0
rs2643133	61833743	0
rs2738252	61833867	0
rs11174816	61833915	0.009
rs7308855	61834323	0.089
rs7298346	61834986	0.11
rs2643132	61835615	0
rs2738250	61835860	0.107
rs2738249	61836288	0
rs2643131	61836294	0
rs2738248	61836384	0
rs2738247	61836619	0
rs2643129	61836874	0
rs2738246	61837084	0
rs10877970	61837421	0.108
rs10877972	61838754	0.108
rs10784342	61839287	0.108
rs1495012	61839901	0
rs11836346	61840232	0.108
rs11174820	61840364	0.117
rs11834212	61840577	0
rs7304733	61840644	0
rs4763054	61841646	0
rs4763052	61841863	0
rs11833019	61842111	0
rs10784343	61842402	0.008
rs728729	61843128	0.096
rs728730	61843138	0.009

rs11174821	61843896	0
rs7307997	61844181	0.417
rs7308008	61844229	0.107
rs17098991	61845506	0.089
rs11174823	61847256	0.008
rs12314824	61849199	0.108
rs11835545	61849214	0.108
rs11174824	61849629	0.017
rs7959001	61851233	0.11
rs11832266	61851444	0.018
rs11832877	61852342	0.108
rs4763062	61806598	0.17

Linkage Disequilibrium Plot for *AVPR1A*



Appendix B

Dodge & Coie's (1987) Aggression Scale

- When teased, strikes back
- Blames others in fights
- Overreacts angrily to accidents
- Teases and name-calls
- Starts fights with peers
- Gets into verbal arguments
- When frustrated, quick to fight
- Breaks rules in games
- Responds negatively when fails
- Uses physical force to dominate
- Gets others to gang up on a peer
- Threatens and bullies others