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Vasopressin, and not Oxytocin, Receptor Gene Methylation is Associated with Individual Differences in Receptive Joint Attention in Chimpanzees (*Pan troglodytes*)

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Abstract

Joint attention (JA) is an important milestone in human infant development and is predictive of the onset of language later in life. Clinically, it has been reported that children at risk for or with a diagnosis of autism spectrum disorder (ASD) perform more poorly on measures of JA compared to neurotypical controls. JA is not unique to humans but has also been reported in great apes and to a lesser extent in more distantly related monkeys. Further, individual differences in JA among chimpanzees are associated with polymorphisms in the vasopressin and oxytocin genes, *AVPR1A* and *OXTR*. Here, we tested whether individual variation in DNA methylation of *OXTR* and *AVPR1A* were associated with performance on JA tasks in chimpanzees. We found that individual differences in JA performance was associated with *AVPR1A* methylation, but not *OXTR* methylation in the chimpanzees. The collective results provide further evidence of the role of *AVPR1A* in JA abilities in chimpanzees. The results further suggest that methylation values for *AVPR1A* may be useful biomarkers for identifying individuals at risk for ASD or related neurodevelopmental disorders associated with impairments in JA abilities.

LAY SUMMARY: This study examines how chimpanzee performance on joint attention tasks relate to the methylation of two genes associated with autism spectrum disorder (ASD). We found that chimpanzees that performed better on one task had lower methylation of the vasopressin receptor gene (*AVPR1A*). This indicates that *AVPR1A* methylation may be a promising ASD biomarker for predicting whether a given individual is at risk for developing impairments in nonverbal social communication.

Joint attention (JA) or joint engagement refers to the dyadic process in which preverbal individuals begin to respond to (receptive joint attention, RJA), and initiate (IJA) nonverbal bids of communication via the use of gaze, gesture and vocalizations (Adamson, 1996; Bates et al., 1975). Typically developing children progress through RJA then IJA skills and studies

have shown that performance in these early JA abilities can predict language abilities at later points in development (Baldwin, 1995; Bottema-Beutel, 2016; Cetincelik et al., 2020; Charman et al., 2000; Morales et al., 2000). A number of studies have also shown that children with or at risk for the development of autism spectrum disorder (ASD) are less inclined to engage in or appropriately develop JA skills compared to neurotypical controls (Bottema-Beutel, 2016; Dawson et al., 2002; Mundy, 2018; Sullivan et al., 2007; Whalen et al., 2006; N. Yirmiya et al., 2006). However, the mechanisms that underlie differences in JA performance between ASD and neurotypical controls remains poorly understood. Furthermore, the role that different genetic and non-genetic factors play on individual variation and the developmental trajectory in JA performance in non-clinical populations of children is also largely unknown.

Two genes repeatedly linked to human social behavior and cognition as well as ASD are oxytocin receptor (*OXTR*) and arginine-vasopressin receptor 1a genes (*AVPR1A*; (Mundy & Bullen, 2021; Skuse & Gallagher, 2011; Wilczyński et al., 2019). Many studies have reported that *OXTR* genotype is related to higher risk of ASD diagnosis, and differences in social behavior and cognition (Skuse et al., 2014; Stavropoulos & Carver, 2013; Tops et al., 2011; M Wade et al., 2014; Wilczyński et al., 2019). Infants with the GG genotype had higher social cognition scores (including JA, empathy, cooperation, and self-recognition) compared to those with the AA or AG genotypes (M Wade et al., 2014). Related, in adults, those with the GG genotype had less difficulty processing social vocal communication (Tops et al., 2011). There have been fewer studies of the role of *OXTR* DNA methylation and ASD or social cognition and behavior, and results have been contradictory; studies have reported both hypermethylation and hypomethylation (Maud et al., 2018; Moerkerke et al., 2021). People diagnosed with ASD had higher methylation of several *OXTR* CpG sites measured from blood samples, and several measured in brain tissue (specifically the temporal cortex) compared to controls (Gregory et al., 2009). Andari and Rilling (2021) found hypermethylation of one CpG site in adults with ASD compared to controls, as well as a relationship between higher methylation and reduced social responsiveness. However, in children, researchers found decreased methylation of *OXTR* exon 1 and exon/intron 2 (Elagoz Yuksel et al., 2016) and at one particular site (in males only; Siu et al., 2021) in those diagnosed with ASD compared to controls.

Though far fewer studies have been published, *AVPR1A* genotypes have also been linked to ASD diagnoses as well as measures of social cognition (Wilczyński et al., 2019). Yirmiva et al. (2006) found that AVPR1A microsatellite haplotypes were associated with a higher risk of ASD and scores on three separate ASD trait measures. Yang et al. (2017) found that polymorphisms in the promotor region of AVPR1A were associated with worse social functioning as measured by multiple ASD social behavior scales. However, other studies showed no such associations (Wilczyński et al., 2019). No studies have examined *AVPR1A* DNA methylation and the relationship with social cognition in humans, nor have studies have examined the relationship with joint attention, specifically, in humans or animal models.

JA abilities are not uniquely human but have been reported in all great apes and, to a lesser extent, in more distantly related primate species. For instance, chimpanzees and other

great apes will follow gaze and pointing gestures to objects and will return objects that are requested from them based on vocal and gestural cues (Clark et al., 2019; Hopkins et al., 2013; Leavens et al., 2008; Leavens & Racine, 2009). Chimpanzees and other great apes will also gesture to foods or objects that are otherwise out of their reach while alternating their gaze between the referent and a human experimenter, though there is some debate regarding nonhuman primates to engage in declarative pointing (Cartmill & Byrne, 2007; Clark et al., 2019; Gretscher et al., 2017; Leavens & Hopkins, 1998; Leavens, Hopkins, et al., 2004; Leavens, Hostetter, et al., 2004; Leavens et al., 2015; Leavens et al., 2005; Liebal et al., 2004; Liszkowski et al., 2004; Liszkowski et al., 2009; MacLean & Hare, 2013; Poss et al., 2006; Tanner & Byrne, 2010; Tomasello, 2008). Indeed, at least two measures of receptive joint attention (RJA) that significantly distinguish between children at risk for or with a diagnosis of ASD from neurotypical controls (herein referred to as the Dawson and Mundy tasks) have been used to assess RJA with chimpanzees (Hopkins et al., 2014a; Hopkins & Latzman, 2021). As in humans, chimpanzees show considerable individual variation in performance on the Dawson and Mundy tasks. Previous studies have reported that individual differences in chimpanzee performance on the Dawson and Mundy tasks are associated with polymorphisms in the vasopressin V1a receptor gene, *AVPR1A* (Hopkins et al., 2014a; Hopkins & Latzman, 2021).

Here, we examined individual differences in RJA performance in chimpanzees in relation to DNA methylation in *AVPR1A* and *OXTR*, the genes encoding the receptors for the neuropeptides vasopressin and oxytocin, respectively. Studies on epigenetics are relatively rare in nonhuman primates, including chimpanzees (Guevara et al., in press; Staes et al., in press). This is unfortunate in light of the fact that chimpanzees have a relatively immature brain at birth (Leigh, 2004) and prolonged period of infancy (Jones, 2011) compared to other primate species, making them ideal models of neurodevelopmental disorders. DNA methylation is a gene regulatory mechanism that is known to mediate long-term stable behavioral differences in response to social environment inputs during early life sensitive periods (Champagne & Curley, 2009; Tost et al., 2015). As defined by Staes et al. (in press), CpG methylation, or DNA methylation comprises the addition of a methyl chemical group to cytosine DNA bases within the context of CpG sites (CpGs), or cytosines next to guanine bases. Methylation can reflect and maintain a gene's transcriptional status by altering transcription factor activity and chromatin structure. Because of the hypothesized role of vasopressin and oxytocin on social cognition and behavior, including joint attention (Francis et al., 2016; Hammock & Young, 2006; LoParo & Waldman, 2014; M. Wade et al., 2014; Zhang et al., 2017), we predicted that methylation in one or both of the receptor genes, *AVPR1A* and *OXTR*, would be associated with RJA performance in the chimpanzees. Specifically, we predicted that altered expression of either *OXTR* or *AVPR1A* (as reflected in lower methylation values) would be positively associated better RJA performance.

In addition, we also examined influence of early social rearing experiences on DNA methylation values for *AVPR1A* and *OXTR*. Previous studies in rodents and rhesus monkeys have reported that newborn offspring that experienced typical or adverse rearing experiences show differential expression of a wide range of genes, which in turn, influence the development of species typical behavior and the expression of some ASD-like behaviors, such as poor social skills and repetitive behaviors (Baker et al., 2017; Dettmer & Suomi,

2014). Within our sample of chimpanzees, there were three cohorts, which included individuals raised by their biological conspecific mothers (MR), those raised in a human nursery (NR) setting with same aged peers for the first three years of life (Bard, 1994) and a few wild-born chimpanzees. A number of previous studies have reported significant differences in social behavior and brain morphology between MR and NR chimpanzees (Bennett et al., 2021; Davenport & Rogers, 1970; Davenport et al., 1973; Hopkins et al., 2020; Menzel et al., 1970; Turner et al., 1969). Given the role that oxytocin and vasopressin play in mammalian social behavior, we hypothesized that MR and NR chimpanzee would differ in DNA methylation values for these two neuropeptides.

Methods

Subjects

Subjects included 54 chimpanzees (27 females, 27 males) from the National Center for Chimpanzee Care (NCCC) at The University of Texas MD Anderson Cancer Center. Blood samples and the magnetic resonance image scans were obtained during the subject's annual physical exam and were collected prior to 2015 when captive chimpanzees were classified as endangered thereby placing limits on the collection of blood samples for purely research purposes. For most chimpanzees, collection of the behavioral data overlapped with the same period of time as the collection of blood samples (average of 0.45 years between collection times). Though 84 chimpanzees were included in the original blood collection, only the 54 with known rearing histories and behavioral data are included here. Age at the time of blood collection ranged from 12 to 59 years old (M = 26.22, SD = 10.05). Within this sample, there were 33 mother-reared and 21 nursery-reared chimpanzees. We defined a nursery-reared (NR) chimpanzee as an individual that was separated from his or her mother within the first 30 days of life due to unresponsive care, injury, or illness (see Bard, 1994; Bard et al., 1992 for details). These chimpanzees were placed in incubators, fed standard human infant formula and cared for by humans until they could sufficiently care for themselves. They were then placed with other infants of the same age until they were three years old (Bard, 1994; Bard et al., 1992). At three years of age, the nursery-reared chimpanzees were integrated into larger social groups of adult and sub-adult chimpanzees. Mother-reared (MR) chimpanzees were not separated from their mother during at least the first 2.5 years of life and were raised in 'nuclear' family groups of conspecifics, ranging in size from 4 to 20 individuals.

DNA extraction, methylation profiling, and data processing

Blood samples were collected from 84 chimpanzees from both NCCC (n=22) and Emory National Primate Research Center (formerly Yerkes; n=62) including 50 females and 34 males between 7 and 59 years of age (M=28.71, SD=11.83). Genomic DNA was extracted from 200 μl of blood samples using the QIAampDNA Mini Kit automated on a QiaCube (Qiagen). DNA concentrations were quantified using a Nanodrop 2000 (Thermo-Fisher Scientific) spectrophotometer. DNA samples were brought to a concentration of ~70 ng/μL. These samples then underwent bisulfite conversion prior to being run on the Illumina Infinium Methylation EPIC array at the Yale Center for Genome Analysis. Data was filtered to remove probes with spectral intensities not significantly different from background levels

and normalized to account for the two probe types on the EPIC array using the illumina GenomeStudio software. In addition, because this array was designed to assay methylation levels at sites in the human genome, analyses were limited to CpG sites expected to also be successfully assayed in chimpanzees (Guevara *et al.* 2020). These CpG sites mapped to the chimpanzee genome (panTro2.1.4) with one or zero mismatches (Needhamsen *et al.* 2017). Raw intensity data for the remaining probes was then converted to beta values (proportion methylation), resulting in 19 and 17 CpG sites for *OXTR* and *AVPR1A* genes, respectively. Only data from 54 of these chimpanzees (with known rearing histories and complete behavioral data) are included in the subsequent analyses.

Receptive Joint Attention (RJA)

Mundy Task: This task was designed to model those used in a previous study of human children by Mundy, Card and Fox (2007). Each chimpanzee received 24 test trials, divided over 4 sessions, with only one 6-trial session performed per day. Prior to beginning the task, the experimenter placed two PVC stations as high and far laterally apart on the cage mesh as possible, but within 1–2 meters of the focal subject. The experimenter positioned themselves in front of the subject an equal distance between the two PVC stations and engaged them in some basic husbandry training task. While the subject was actively engaged with the experimenter, the experimenter stopped interacting with the subject and pointed (full arm extended and maintained throughout the trial) and looked toward one of the PVC stations (the cued PVC) and said the chimpanzee's name with increasing emphasis. If the subject looked at, oriented toward, or touched the cued PVC station during this time, they received a "1", indicating a correct response. If the subject did not look at, orient toward, or touch the cued PVC, or if they instead looked at, oriented toward or touched the non-cued PVC pipe, then they received a score of "0" for that trial, indicating an incorrect response. This process was repeated for all six trials within a session, with each trial separated by the experimenter re-engaging the subject with the basic husbandry training task. The experimenter randomly alternated which of the PVC stations was the cued stimulus. The dependent measure was the proportion of correct responses across the 24 trials.

Dawson Task: The methods for assessing RJA for this task have been described in detail elsewhere (Hopkins et al., 2014b). Briefly, at the onset of each trial, a human experimenter would engage in basic husbandry training activities with the focal subject. When the experimenter sensed that the focal chimpanzee was engaged and facing them, they would stop their action and initially look over the shoulder of the subject for 5 s, as if there were an object behind them. At the end of this cue, the chimpanzee's behavior was recorded for 15 s. If they looked behind them, they were given a score of 3 and the trial ended. If the focal chimpanzee subject did not look behind them, the experimenter re-engaged the subject in husbandry training behavior. When the experimenter judged the subject to be engaged and facing them, they stopped and again looked over the focal subject's shoulder and pointed as if there were an object behind the ape. Following this cue, the chimpanzee was again observed for 15 s, and if they looked behind them, they were given a score of 2 and the trial ended. As before, if the chimpanzee did not look behind them, the experimenter re-engaged the chimpanzee in husbandry training behavior. When the experimenter again sensed that the chimpanzee was engaged, they stopped and now looked over the focal subject's shoulder,

pointed and vocally prompted the chimpanzee to an object behind them. Following this cue, the chimpanzee's response was recorded for 15 s and if they looked behind them, they were given a score of 1 and the trial ended. If the subject failed to look behind them at the end of this phase of the trial, they were given a score of 0. Each chimpanzee received 4 trials and the trials were administered across different days. We summed their performance across the 4 test trials to create a single composite score that ranged between 0 and 16 with higher values indicating better performance.

Statistical analysis

Statistical analyses were performed using SPSS. The methylation values for each gene were then (1) compared between sexes and rearing groups, and (2) correlated with each RJA performance measure as well as their average performance for both measures. Because the two JA tasks were on different scales of measurement, we converted them to standardized *z*-scores and then computed their average to derive the composite performance score (RJA_Mean). When testing for differences in methylation between rearing conditions and sexes, we performed multivariate analyses of covariance while controlling for age at the time of blood collection and genetic relatedness. Genetic relatedness was computed based on available pedigree information and reflected how related each subject is to all other chimpanzees within the colony that they were born. See Mulholland et al. (2020) for the methods used to calculate the relatedness coefficients.

Results

Sex and Rearing Effects on OXTR and AVPR1A Methylation

We identified 19 and 17 CpG sites for *OXTR* and *AVPR1A* genes, respectively. The specific CpG sites are shown in Tables 1 and 2. We first evaluated the influence of sex and rearing experiences on the methylation values for the *AVPR1A* and *OXTR* genes. For *AVPR1A*, the MANCOVA revealed no significant main effect for sex $F(17,32)=1.437$ $p=0.184$, rearing $F(17,32)=1.362$ $p=0.220$, nor a sex by rearing interaction $F(17,32)=0.782$ $p=0.700$. For *OXTR*, the MANCOVA also revealed no significant main effect for rearing $F(19,30)=0.541$ $p=0.918$, sex $F(19,30)=1.283$ $p= 0.264$, nor a sex by rearing interaction $F(19,30)=1.208$ $p=0.314$. Therefore, sex and rearing were not included as covariates for subsequent analyses.

Associations Between Receptive Joint Attention, AVPR1A and OXTR Methylation

For *AVPR1A*, performance on the Dawson task was negatively correlated with methylation values of 8 CpG sites and positively correlated with methylation values of 1 CpG site. Mean_RJA was negatively correlated with methylation values of 5 CpG sites and positively correlated with methylation values of 1 CpG site. Performance on the Mundy task, however, was only positively correlated with methylation of one CpG site. In contrast, for *OXTR*, there were no significant correlations between performance on the JA tasks and methylation values. See Table 3 for specific CpG sites, correlation coefficients, and adjusted p-values (5% FDR; calculated using the R function *p.adjust*).

Discussion

Contrary to our hypothesis, results from this study revealed no effect of sex or rearing on methylation values for vasopressin and oxytocin receptor genes (*AVPR1A* and *OXTR*). However, as predicted, we found that performance on the two joint attention tasks and average RJA performance were significantly associated with *AVPR1A* CpG site methylation. Specifically, we found that performance on the Dawson task was negatively correlated with methylation values on 9 *AVPR1A* CpG sites including: cg09208611, cg12807275, cg16668728, cg24501701, cg09040797, cg10862431, cg12516059, cg21164131, and cg27032502, and positively correlated with methylation values of cg13631391. Chimpanzees that performed better on the Dawson measure had lower *AVPR1A* methylation for all but one of these CpG sites (cg13631391). Poor performance on the Dawson task is associated with higher *AVPR1A* methylation at these 9 sites, and increased methylation (or hypermethylation) can lead to gene inactivation or reduced gene expression. Unfortunately, we cannot determine the causal direction of this relationship; this could mean that higher methylation/reduced *AVPR1A* gene expression leads to the development of poor RJA skills or that poor RJA skills leads to hypermethylation, or that some other variable could cause changes in both RJA and *AVPR1A* methylation. However, this is consistent with previous studies in chimpanzees that have reported significant associations between performance on the Dawson joint attention task and polymorphisms in the *AVPR1A* gene (Hopkins et al., 2014a). Specifically, chimpanzee males that were homozygous for a sequence deletion in the 5' flanking region of *AVPR1A* had poorer RJA skills (Hopkins et al., 2014a). Additionally, chimpanzees who performed poorly on the Dawson task had atypical (more rightward) gray matter volume asymmetries in the posterior superior temporal sulcus, a brain region known to be important for the processing of socially-relevant information (Hopkins, et al., 2014b). Thus, collectively, these findings strongly implicate the vasopressin pathway in receptive joint attention, as quantified by the Dawson task alone. Interestingly, there were far fewer associations between *AVPR1A* methylation and a second measure of RJA, the Mundy task. Like the Dawson task, performance on the Mundy task was positively associated with methylation values at cg13631391, but there were no other significant relationships. Unfortunately, there are no published papers on *AVPR1A* methylation that could help us explain why this one particular CpG site (cg13631391, genome location 63544945) would have the opposite relationship with RJA performance than the others. Additionally, in light of the fact that the Mundy and Dawson task are both considered measures of receptive joint attention, one would expect that there would be (1) a significant positive relationship with each other [there is, in fact, a moderate positive correlation: $r(49) = 0.418$, $p = 0.002$], and (2) more consistent associations between performance and the *AVPR1A* methylation values.

The lack of a stronger positive correlation between performance on the Mundy and Dawson tasks may reflect subtle differences in the two tasks. While both tasks require the chimpanzees to respond to communicative cues of a researcher, the scoring method and cues utilized are different. The Mundy task requires the chimpanzees to either look, orient, or point towards a cued target object on their immediate left or right (as indicated by the researcher's simultaneous gaze, pointing and vocal cues). Higher scores indicate the

number of trials the chimpanzees made correct responses. The Dawson task requires the chimpanzees to look behind them in the direction of the researcher's gaze, or their gaze paired with either pointing, or pointing and vocalizing. Higher scores on the Dawson task indicate that chimpanzees required fewer cues to respond correctly (i.e. gaze following only would receive the highest score). The middle and low scores (requiring the researcher to point or point and vocalize, or not eliciting a correct response from the chimpanzee) are more congruent with scoring of the Mundy task. Therefore, it is more difficult to obtain a high score on the Dawson task compared to the Mundy task. Chimpanzees with higher Dawson scores (more likely to respond with fewer researcher cues) are more likely to have lower *AVPR1A* methylation across several CpG sites. Also, if the Mundy task included trials where cued objects were behind the chimpanzees, then perhaps the relationship with *AVPR1A* methylation would be more congruent with that of the Dawson performance.

Surprisingly, there were no significant relationships between JA performance (on any task) and *OXTR* methylation at any CpG sites. This is contrary to what has been found for humans, with several studies reporting either hyper- and hypomethylation of OXTR related to measures of social cognition (depending on age, sex, and ASD diagnosis (Andari & Rilling, 2021; Elagoz Yuksel et al., 2016; Maud et al., 2018; Moerkerke et al., 2021; Siu et al., 2021). Although we found no relationships between *OXTR* methylation and JA performance, it is possible that these relationships would be found in (1) a chimpanzee sample with more impaired JA abilities, and (2) that *OXTR* genotype may moderate the relationship between OXTR methylation and JA. Rijlaarsdam et al. (2017) examined the associations of OXTR methylation (averaged across 3 CpG sites: cg02192228, cg04523291, cg15317815 located within the CpG island), *OXTR* genotype, and prenatal stress exposure with ASD-related traits in children. They found no association between *OXTR* methylation and ASD traits or *OXTR* genotype and ASD traits when examined separately, however, there was a significant genotype by methylation interaction. Specifically, there was a stronger relationship between methylation and overall social responsiveness, social communication problems, social cognition, and ASD mannerisms for children with particular *OXTR* alleles (homozygous G-allele but not A-allele carriers; Rijlaarsdam et al., 2017). Future research should determine whether *OXTR* genotype moderates the relationship between JA performance and OXTR methylation in chimpanzees, and specifically examine these same three sites identified in children.

There are several limitations to this study. First, measures of methylation were obtained from blood samples which are not cell specific. That stated, previous studies in chimpanzees have reported that methylation values for the *DRD2* gene obtained from blood correlate significantly and positively with methylation values obtained from prefrontal cortex and the cerebellum (Staes et al., in press). Second, the sample size was small and future studies would benefit from a larger cohort of subjects. In addition, although we had equal numbers of females and males, our sample was not comprised of equal numbers of mother- and nursery-reared individuals (female MR n=13, NR n=14; male MR n=20, NR n=7). Lastly, because of the correlational nature of the study, it is not clear whether performance on the JA tasks drives changes in *AVPR1A* methylation or vice versa.

Implications for Autism

Caveats and limitations aside, we believe the findings from this study have some implications for research on Autism Spectrum Disorder (ASD). According to the DSM-5, ASD is characterized by impairments in three broad behavioral categories or phenotypes, including (1) stereotyped or repetitive behaviors, (2) impairments in social behavior, and (3) socio-communicative deficits, particularly early in development. Because ASD is a neurodevelopmental disorder, there has been considerable effort devoted to identifying early behavioral and biological markers (including candidate genes) that predict whether a given individual may be at risk for the development of ASD (Constantino et al., 2017; Jones & Klin, 2013; Lord & Spence, 2006; Melke, 2008; Mundy, 2018; Osterling et al., 2002; Zwaigenbaum et al., 2005). The primary measure of socio-communicative impairment in preverbal children has been JA. To date, both the *OXTR* and *AVPR1A* receptor genes have been identified as risk genes for ASD (Hammock & Young, 2006; LoParo & Waldman, 2014). Our results suggest that rather than focus entirely on genetic polymorphisms, *AVPR1A* methylation may potentially serve as another biomarker for identifying individuals at risk for the development of ASD.

In summary, individual variation in *AVPR1A* methylation, but not OXTR methylation, was associated with measures of receptive joint attention, further implicating this gene in non-verbal, socio-communicative functions. Future studies on epigenetic processes, and interactions with genotype, in chimpanzees will provide invaluable data on how experiences shape the brain and cognition in primates, including humans.

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Table 1.

AVPR1A CpG site information.

	Site	Chromosome	Position	Relation to Island	Gene Region
1	cg04827692	chr12	63543831	Island	1stExon
2	cg09208611	chr12	63544319	Island	1stExon
3	cg10931900	chr12	63540400	N_Shelf	3’UTR
4	cg12807275	chr12	63543292	N_Shore	Body
5	cg16352140	chr12	63544015	Island	1stExon
6	cg16668728	chr12	63544013	Island	1stExon
7	cg23335356	chr12	63547716	S_Shelf	TSS1500
8	cg24501701	chr12	63544040	Island	1stExon
9	cg26727693	chr12	63544175	Island	1stExon
10	cg09040797	chr12	63544768	Island	1stExon;5’UTR
11	cg10862431	chr12	63544783	Island	1stExon;5’UTR
12	cg12516059	chr12	63545288	S_Shore	1stExon;5’UTR
13	cg13631391	chr12	63544945	Island	1stExon;5’UTR
14	cg19987210	chr12	63544752	Island	1stExon;5’UTR
15	cg21164131	chr12	63544923	Island	1stExon;5’UTR
16	cg23549160	chr12	63545956	S_Shore	1stExon;5’UTR
17	cg27032502	chr12	63544881	Island	1stExon;5’UTR

Table 2.

OXTR CpG site information.

	Site	Chromosome	Position	Relation to Island	Gene Region
1	cg00078085	chr3	8810592	Island	5’UTR
2	cg00247334	chr3	8811543	S_Shore	TSS1500
3	cg00385883	chr3	8808259	N_Shore	Body
4	cg02192228	chr3	8809536	Island	Body
5	cg03257388	chr3	8809213	Island	Body
6	cg03710862	chr3	8811728	S_Shore	TSS1500
7	cg03987506	chr3	8810549	Island	5’UTR
8	cg04523291	chr3	8809501	Island	Body
9	cg11171527	chr3	8810206	Island	5’UTR
10	cg14483142	chr3	8811758	S_Shore	TSS1500
11	cg15317815	chr3	8809306	Island	Body
12	cg17036624	chr3	8811601	S_Shore	TSS1500
13	cg19619174	chr3	8810139	Island	5’UTR
14	cg25085537	chr3	8811739	S_Shore	TSS1500
15	cg26455676	chr3	8797459	OpenSea	Body
16	cg27501759	chr3	8809715	Island	Body
17	cg09353063	chr3	8811092	Island	1stExon;5’UTR
18	cg17285225	chr3	8811004	Island	1stExon;5’UTR
19	cg23391006	chr3	8811279	Island	1stExon;5’UTR

Table 3.

Partial Correlation Coefficients between each CpG site and each JA measure (df=50). All p-values are adjusted based on a 5% FDR.

	Mean_RJA		Mundy		Dawson	
AVPR1A CpG Site	**r**	**adj. p-value**	**r**	**adj. p-value**	**r**	**adj. p-value**
cg04827692	−0.233	0.171	−0.047	0.786	−0.304	0.070
cg09208611	−0.286	0.085	−0.052	0.778	**−0.378**	**0.031***
cg10931900	−0.24	0.164	−0.024	0.864	−0.333	0.048
cg12807275	**−0.378**	**0.031***	−0.159	0.370	**−0.425**	**0.020***
cg16352140	0.219	0.184	0.234	0.171	0.13	0.464
cg16668728	−0.298	0.071	−0.133	0.464	−0.329	**0.048***
cg23335356	−0.031	0.847	0.095	0.555	−0.124	0.464
cg24501701	−0.319	0.056	−0.127	0.464	**−0.365**	**0.034***
cg26727693	−0.306	0.070	−0.228	0.171	−0.262	0.120
cg09040797	**−0.384**	**0.031***	−0.125	0.464	**−0.462**	**0.017***
cg10862431	−0.302	0.070	−0.118	0.478	**−0.347**	**0.044***
cg12516059	**−0.341**	**0.044***	−0.132	0.464	**−0.393**	**0.029***
cg13631391	**0.477**	**<0.001***	**0.331**	**0.048***	**0.429**	**0.017***
cg19987210	0.219	0.184	0.113	0.494	0.23	0.171
cg21164131	**−0.363**	**0.034***	−0.174	0.322	**−0.391**	**0.029***
cg23549160	−0.173	0.322	0.034	0.842	−0.283	0.086
cg27032502	**−0.346**	**0.044***	−0.106	0.517	**−0.422**	**0.020***

	Mean_RJA		Mundy		Dawson	
OXTR CpG Site	**r**	**adj. p-value**	**r**	**adj. p-value**	**r**	**adj. p-value**
cg00078085	0.085	0.810	−0.084	0.810	0.196	0.489
cg00247334	−0.071	0.859	−0.049	0.905	−0.064	0.859
cg00385883	0.104	0.810	0.21	0.431	−0.019	0.963
cg02192228	−0.14	0.726	−0.286	0.234	0.029	0.963
cg03257388	0.008	0.990	−0.099	0.810	0.093	0.810
cg03710862	0.079	0.821	0.168	0.606	−0.021	0.963
cg03987506	−0.057	0.870	−0.229	0.363	0.104	0.810
cg04523291	−0.129	0.762	−0.256	0.333	0.02	0.963
cg11171527	−0.028	0.963	−0.045	0.908	−0.004	0.990
cg14483142	−0.248	0.333	−0.338	0.114	−0.089	0.810
cg15317815	−0.042	0.908	0.109	0.810	−0.153	0.663
cg17036624	−0.101	0.810	−0.192	0.490	0.008	0.990
cg19619174	−0.138	0.726	−0.249	0.333	0.002	0.990
cg25085537	−0.361	0.114	−0.239	0.338	−0.338	0.114

	Mean_RJA		Mundy		Dawson	
AVPR1A CpG Site	**r**	**adj. p-value**	**r**	**adj. p-value**	**r**	**adj. p-value**
cg26455676	−0.238	0.338	−0.352	0.114	−0.063	0.859
cg27501759	−0.392	0.114	−0.358	0.114	−0.285	0.234
cg09353063	0.164	0.610	0.09	0.810	0.168	0.606
cg17285225	−0.062	0.859	−0.213	0.431	0.084	0.810
cg23391006	−0.295	0.234	−0.415	0.114	−0.094	0.810

* indicates significant correlations