Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans

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Pair-bonding has been suggested to be a critical factor in the evolutionary development of the social brain. The brain neuropeptide arginine vasopressin (AVP) exerts an important influence on pair-bonding behavior in voles. There is a strong association between a polymorphic repeat sequence in the 5′ flanking region of the gene (avpr1a) encoding one of the AVP receptor subtypes (V1aR), and proneness for monogamous behavior in males of this species. It is not yet known whether similar mechanisms are important also for human pair-bonding. Here, we report an association between one of the human AVPR1A repeat polymorphisms (RS3) and traits reflecting pair-bonding behavior in men, including partner bonding, perceived marital problems, and marital status, and show that the RS3 genotype of the males also affects marital quality as perceived by their spouses. These results suggest an association between a single gene and pair-bonding behavior in humans, and indicate that the well characterized influence of AVP on pair-bonding in voles may be of relevance also for humans.

monogamy | neuropeptide | polymorphism | social behavior

Primate social organization is often characterized by bonded relationships, and recent analyses suggest that it may have been the particular demands for pair-bonding behavior that triggered the evolutionary development of the primate social brain (1). The brain neuropeptide arginine vasopressin (AVP), acting through the receptor subtype V1aR, plays a key role in the regulation of pair-bonding behavior in male rodents, as revealed by a series of elegant studies on closely related vole species, i.e., montane voles (Microtus montanus), meadow voles (Microtus pennsylvanicus), and prairie voles (Microtus ochrogaster) (2). In prairie voles, which in contrast to montane and meadow voles are socially monogamous and highly social, pair-bond formation and related behaviors are facilitated by AVP and prevented by a V1aR antagonist (3). Supporting the theory that the striking difference in pair-bonding between monogamous and nonmonogamous voles is related to the influence of AVP on this behavior, the neuroanatomical distribution of V1aR differs considerably between these vole species (4) and is associated with sexual and social fidelity among prairie voles (5). Moreover, partner preference is enhanced in the nonmonogamous meadow vole when the V1aR density is increased in relevant brain areas by using viral vector gene transfer (6). Although there are no major differences in the coding sequence of the gene encoding V1aR (avpr1a) between prairie, montane or meadow voles, the former species displays a 428-base pair sequence in the 5′ flanking region that is not found in the latter two species. When the avpr1a of the prairie vole, including the sequence in the 5′ region, is transgenically inserted into the nonmonogamous species mouse (7), more pronounced social behavior, similar to that displayed by prairie voles, is generated. Furthermore, variation in the 5′ flanking region of prairie vole avpr1a affects brain expression of the gene and alters intraspecific variation in partner preference (8).

Human AVPRIA is situated on chromosome 12q14–15 (9). Whereas there is no sequence in the human AVPRIA 5′ flanking region homologous to the one found in prairie voles, humans do have three repetitive sequences in this region that are polymorphic: A (GT)25 dinucleotide repeat, a complex (CT)4-TT-(CT)8-(GT)24 repeat (RS3), and a (GATA)14 tetranucleotide repeat (RS1) (10). Although as yet not consistently replicated, previous studies have revealed associations between AVPRIA repeat polymorphisms and autism (11–13), age at first sexual intercourse (14), and altruism (15), suggesting that these repetitive sequences may have an impact on human social behavior.

The aim of this study was to investigate whether variability in the 5′ flanking region of AVPRIA affects pair-bonding behavior in humans as it does in prairie voles. To this end, the three repeat polymorphisms of the AVPRIA were genotyped in adult men and women from the Twin and Offspring Study in Sweden (TOSS), comprised of 552 same-sex twin-pairs and their spouses/partners (16). All subjects were assessed with respect to various indices of the quality of the marital relationship, including a new scale—the Partner Bonding Scale (PBS)—which is comprised of items that correspond to the behavioral patterns observed when measuring features of pair-bonds among nonhuman primates.

Results

The allele and genotype distributions of the three repeat polymorphisms (RS1, RS3, and GT25) were similar to what has been reported in previous studies (10, 11, 17) and did not deviate from Hardy–Weinberg equilibrium. After correction for multiple tests, there was a significant global P value for an association between the RS3-repeat polymorphism and the outcome of the PBS for men (P < 0.01 after a Bonferroni correction of the six tests), but not for women (Table 1). No associations were found for the other AVP1A polymorphisms. When comparing the mean scores of the PBS for each RS3 allele (Table 2), this value was found to be significantly lower for men carrying allele 334 than for those not carrying this allele (F1,130 = 16.35, P < 0.0001, 0.01 after a Bonferroni correction of the six tests).
partner bonding scale in men.

Table 1. Association between the different microsatellite polymorphisms in the AVPR1A 5’ flanking region and the Partner Bonding Scale

<table>
<thead>
<tr>
<th>Allele</th>
<th>Freq</th>
<th>Percent</th>
<th>Mean</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT25</td>
<td>330</td>
<td>92</td>
<td>47.6 (7.18)</td>
<td>1.37</td>
<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td>RS1</td>
<td>330</td>
<td>92</td>
<td>47.6 (7.18)</td>
<td>1.37</td>
<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td>RS3</td>
<td>330</td>
<td>92</td>
<td>47.6 (7.18)</td>
<td>1.37</td>
<td>0.21</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Only genotypes for which \( n > 10 \) were included in the analyses.

Discussion

The results from the current study suggest an association between the AVPR1A polymorphism and human pair-bonding behavior possibly analogous to that reported for voles (8). One of the most common RS3 alleles, the allele 334, was associated with perceived partner bonding in men as assessed by using the PBS. This association could be detected also by assessing marital problems and marital status in men, and the perception of the quality of the marital relationship expressed by their spouses. That an association between the studied gene and items reflecting marital quality, as perceived by partners, was significant is consistent with the fact that the influence of vasopressin on social behavior is more prominent in male than in female voles (20).

Although the functional importance of the RS3 polymorphism of the AVPR1A remains to be clarified, an association between the length of the RS3 repeat and the amount of hippocampal mRNA in human postmortem tissue has been reported (15). Moreover, a recent study in healthy subjects suggests that the 334 allele is associated with increased activation of amygdala, a brain region known to be of importance for pair-bonding behavior (17). The conclusion of our study (that the 334 allele of the RS3 polymorphism influences brain function) is well in line with previous observations.

The possible influence of AVP on social interactions has led researchers to suggest an involvement of this transmitter in conditions characterized by social deficits, for example, autism and autism-related conditions. This theory has gained support from studies assessing the possible association between AVPR1A and risk for autism (11–13) and other traits related to interpersonal relationships (15). Although it is difficult to compare the results of these studies to those of our study, it is of interest to note that one of these studies suggests the 334 allele to be over-transmitted to subjects with autism (11). The observation that a gene variant, which according to our data, is negatively associated with the ability to interact within a relationship, may enhance the risk for a condition characterized by impaired social impairments of social relatedness and communication is obviously noteworthy.

The effect size \( (d) \) for the influence of the studied allele on PBS scores when comparing men who carry one or two 334 alleles with those who do not carry any was 0.27. This is comparable with what has been reported in large metaanalyses of the association between a DRD4 polymorphism and the personality trait novelty seeking \( (d = 0.32) \) (19) and that between a serotonin transporter polymorphism and neuroticism \( (d = 0.23) \) (20), despite the fact that the outcome of the PBS, unlike
novelty seeking and neuroticism to some extent is influenced not only by the informant but also by his/her partner.

It is notable that an association was found between the RS3 repeat of the AVPR1A and indices of pair-bonding behavior in a cohort in which all subjects had been married or cohabiting for at least five years. Tentatively, such an association would be even stronger in a population also comprising subjects not involved in any long-term romantic relationships. It would also be of importance to assess the possible influence of this polymorphism on measures of pair-bonding that are more objective than self-report, such as proneness for cohabiting versus living alone, marriage, and divorce. However, of some interest in this context is our observation that men that were homozygous for the 334 allele were more likely to be unmarried than other men, despite the fact that the cohabiting individuals in our sample had been in a relationship persisting for at least five years and that in the vast majority of all of these couples, both individuals were biological parents to an adolescent child, ranging in age from 11-22-years-old. This finding is in line with the observation that unmarried men displayed lower scores on the PBS (see Materials and Methods) and may tentatively reflect a lower degree of commitment in those being unmarried.

The relatively small effect size of the AVPR1A polymorphism on traits tentatively reflecting pair-bonding in males observed in this study clearly does not mean that this polymorphism may serve as a predictor of human pair-bonding behavior on the individual level. However, by demonstrating a modest but significant influence of this gene on the studied behavior on the group level, we have provided support for the assumption that previous studies on the influence of the gene coding for V1aR on pair-bonding in voles are probably of relevance also for humans.

Materials and Methods

Subjects. The study consisted of 552 twin pairs and their spouses from the second cohort of the Twin and Offspring Study in Sweden (TOSS), a two-cohort study of twin parents, one adolescent child, and the spouse/partner, for which detailed measures of parent-child relationships, marital relationships, personality, attachment style, and the mental health of all study participants were collected (16). Participants were mostly middle class and born between 1944 and 1971. Consistent with the population of Sweden, the vast majority were Caucasian. A more detailed description of the sample is available in a previous article by Neiderhiser and coworkers (16). The same-sex twins included in the study were required to have a relationship of at least five years with their partner; whereas 82% were married, 18% were cohabiting but unmarried. For simplicity, both married and unmarried cohabiting individuals are referred to as “husband,” “wife,” or “spouse.” The twins and their spouses were first sent a questionnaire that was followed by a home visit, during which additional questionnaires were administered. DNA was extracted from mouthwash samples that were collected by using a DNA self-collection kit. Zygosity was determined primarily by genotyping. There were 238 monozygotic (MZ) pairs

Table 3. Effect of 0, 1 or 2 334 alleles on male reports on the Partner Bonding Scale, marital crisis, and marital status

<table>
<thead>
<tr>
<th>Measure</th>
<th>Number of 334 alleles</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean score for the Partner Bonding Scale in the three groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner Bonding Scale</td>
<td></td>
<td>48.0 (6.50)</td>
<td>46.3 (6.16)</td>
<td>45.5 (6.71)</td>
<td>2, 143</td>
<td>8.40</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Table 4. Association between 334 alleles in men and their wives’ reports of marital qualities

<table>
<thead>
<tr>
<th>Quality</th>
<th>No 334 (mean)</th>
<th>One or two 334 (mean)</th>
<th>β</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affectional expression</td>
<td>Unadjusted</td>
<td>18.0 (2.99)</td>
<td>17.4 (2.92)</td>
<td>-0.64</td>
<td>1, 113</td>
<td>10.08</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>-</td>
<td>-0.39</td>
<td>1, 111</td>
<td>4.30</td>
</tr>
<tr>
<td>Dyadic consensus</td>
<td>Unadjusted</td>
<td>65.4 (8.11)</td>
<td>63.9 (8.57)</td>
<td>-1.46</td>
<td>1, 117</td>
<td>6.92</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>-</td>
<td>-0.82</td>
<td>1, 115</td>
<td>2.46</td>
</tr>
<tr>
<td>Dyadic cohesion</td>
<td>Unadjusted</td>
<td>19.5 (4.34)</td>
<td>18.9 (4.10)</td>
<td>-0.60</td>
<td>1, 116</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>-</td>
<td>-0.20</td>
<td>1, 114</td>
<td>0.53</td>
</tr>
<tr>
<td>Dyadic satisfaction</td>
<td>Unadjusted</td>
<td>43.3 (3.14)</td>
<td>43.2 (2.92)</td>
<td>-0.12</td>
<td>1, 111</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Mean, Mean value on the outcome for the different DAS Scales for wives with standard deviation within brackets. Adjusted, Analysis with the Partner Bonding Scale included as a covariate. The category of subjects not carrying any 334 allele was used as reference group when constructing the regression estimates (β). Analyses of adjusted values were only performed for the scales that were significantly associated with the 334 allele in the unadjusted analysis.
and 314 dizygotic (DZ) pairs. In total, 2,186 adult individuals were included in the study, of which 1,899 provided usable DNA samples.

**Partner Bonding Scale.** The pair bond is a critical element in the study of the evolution of primatc social organization (21). Pair bonds among nonhuman primates are generally assessed by measures of partner specific affiliative interaction, proximity, and reciprocity between two individuals (22–24). Furthermore, the strength and stability of the bond is related to its persistence through time (25). In accordance with the behavioral domains observed when studying pair-bonding among nonhuman primates, items were collected from the DAS (19), a frequently used assessment of the quality of marital relationships and similar dyads, the Support Seeking and Giving (SSG) (26) assessment measuring subjects’ engagement with other people, and the Marital Instability Scale (MIS) (27). Partner specific affiliative interaction was measured by the occurrence of partner exclusive actions (for example, “How often do you kiss your mate?”). Proximity measures, which in nonhuman primates are measured as the amount of spatial closeness between two individuals, were assessed by two types of items: The proband’s experiences of closeness to other people (for example, “I don’t like when other people come too close to me”) and items concerning the proband’s motivation to spend time together with their spouse (for example, “How often are you and your partner involved in common interests outside the family?”). Because one requirement for inclusion in the TOSS dataset was that the adult individuals were part of a dyadic relationship that had persisted for at least five years, no information about the final length of the pair-bonds were available. Instead the proband’s reports of their attitudes toward the stability of the relationship (for example, “Have you discussed a divorce or separation with a close friend?”) were used as individual indicators of future persistence of the relationship. No relevant measures of reciprocity could be found in the TOSS dataset. Thus, of a total of 49 items, 18 questions (7 DAS, 10 SSG, and 1 MIS) were considered relevant measures of human pair-bonding. A factor analysis was performed, and items with loadings <0.4 on the first principal component were excluded [see supporting information (SI) Table S1] resulting in the final PBS, which were created as the sum of 13 items (7 DAS, 5 SSG, and 1 MIS), with scores ranging from 5 to 66. The reliability, as measured with Cronbach’s alpha, was 0.79. Validity estimates of the PBS and the original subscales of the validated questionnaires showed plausible patterns of moderate to high correlation coefficients. These results were confirmed by our findings on known-groups validity, which showed significant differences between married and nonmarried subjects (F1,101 = 28.28, P < 0.0001), with nonmarried subjects scoring lower on the PBS. We also observed that subjects that had experienced during the last year marital crisis/divorce of their divorce scored significantly lower on the PBS than those who had not (F1,162 = 186.22, P < 0.0001).

**Microsatellite Genotyping.** The GT25 repeat polymorphism was amplified with primers 5′-TGTAGAGACAAAGCTGTCC-3′ (forward) and 5′-TGGGTTTAA- AAGTTACCCG-3′ (reverse), the RS3 repeat polymorphism was amplified with primers 5′-TCTCTGAGAGTTAAGTG-3′ (forward) and 5′-gttcttCTT- GGAAGAAGCTTTAGG-3′ (reverse) (11, 12, 17). The fluorescently labeled DNA fragments were analyzed by size with automated capillary electrophoresis by using an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

**Statistical Analysis.** Statistical associations between the continuous and categorical predictors on the one hand and continuous and binary criteria on the other were estimated by using Generalized Linear Mixed Effects Models (GLMM). As earlier studies have shown that the effects of vasopressin on pair-bonding behavior differ between sexes (20), all analyses were performed for men and women separately. To take the correlated data structure into account and to avoid estimation problems, different variance-covariance matrices were modeled for monogygotic twins, spouses to monogygotic twins, dizygotic twins, and spouses to dizygotic twins. Each of these four groups had a cluster size of n = 2. The correlations between individuals in these groups were calculated by using R-side random effects with an unstructured variance-covariance matrix. The model for continuous outcomes assumed normal distribution of residuals with an identity link function between the predictor term and the criterion. Dichotomous outcomes were assumed to be binary distributed with a logit link function. The parameters were estimated based on the residual log pseudo-likelihood (RSPL), which is equivalent to restricted maximum likelihood (31).

All statistical analysis was performed by using the Statistical Analysis System (SAS), Version 9.1.3 (32), and generalized linear mixed effects models were implemented by using the PROC GLIMMIX procedure.

**ACKNOWLEDGMENTS.** This project was supported by National Institute of Mental Health Grant R01MH54610, Bank of Sweden Tercentenary Foundation Grant J2004–0036.1, and a postdoctoral fellowship sponsored by the Brain Foundation, Sweden (to L.W.).