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Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA

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Human altruism is a widespread phenomenon that puzzled evolutionary biologists since Darwin. Economic games illustrate human altruism by showing that behavior deviates from economic predictions of profit maximization. A game that most plainly shows this altruistic tendency is the Dictator Game. We hypothesized that human altruistic behavior is to some extent hardwired and that a likely candidate that may contribute to individual differences in altruistic behavior is the arginine vasopressin 1a (*AVPR1a*) receptor that in some mammals such as the vole has a profound impact on affiliative behaviors. In the current investigation, 203 male and female university students played an online version of the Dictator Game, for real money payoffs. All subjects and their parents were genotyped for *AVPR1a* RS1 and RS3 promoter-region repeat polymorphisms. Parents did not participate in online game playing. As variation in the length of a repetitive element in the vole AVPR1a promoter region is associated with differences in social behavior, we examined the relationship between RS1 and RS3 repeat length (base pairs) and allocation sums. Participants with short versions (308–325 bp) of the *AVPR1a* RS3 repeat allocated significantly (likelihood ratio = 14.75, $P = 0.001$, df = 2) fewer shekels to the 'other' than participants with long versions (327–343 bp). We also implemented a family-based association test, UNPHASED, to confirm and validate the correlation between the AVPR1a RS3 repeat and monetary allocations in the dictator game. Dictator game allocations were significantly associated with the RS3 repeat (global P value: likelihood ratio $\chi^2 = 11.73$, df = 4, $P = 0.019$). The association between the AVPR1a RS3 repeat and altruism was also confirmed using two self-report scales (the Bardi–Schwartz Universalism and Benevolence Value-expressive Behavior scales). RS3 long alleles were associated with higher scores on both measures. Finally, long *AVPR1a* RS3 repeats were associated with higher *AVPR1a* human post-mortem hippocampal messenger RNA levels than short RS3 repeats (one-way analysis of variance (ANOVA): $F = 15.04$, $P = 0.001$, df = 14) suggesting a functional molecular genetic basis for the observation that participants with the long RS3 repeats allocate more money than participants with the short repeats. This is the first investigation showing that a common human polymorphism, with antecedents in lower mammals, contributes to decision making in an economic game. The finding that the same gene contributing to social bonding in lower animals also appears to operate similarly in human behavior suggests a common evolutionary mechanism.

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Economic games provide a method for observing human behavior in the laboratory that has many advantages over the standard self-report questionnaires (Camerer & Fehr 2003). Games recreate social interactions in the laboratory using real money payoffs and thus engage people in 'put your money where your mouth is' decisions. A well-defined game also provides the benefits of quantifiability, replicability and

comparability across participants and therefore constitutes a more reliable tool for measuring social decision making.

A robust body of experimental evidence based on laboratory games shows that human behavior deviates from economic predictions of profit maximization. A game that best shows this incongruity is the 'dictator game'. The first player or 'Dictator' makes a unilateral decision regarding the distribution of a fixed sum of money between herself and the second player, the 'Recipient'. Because the recipient is completely powerless, the dictators are unconstrained by fear of reprisal or other strategic considerations, and their allotment can be seen as a measure of pure altruism (Forsythe *et al.* 1994; Kahneman *et al.* 1986). Indeed, the dictator game is the most prominent paradigm used by economists to test the existence of altruism (Bolton *et al.* 1998; Camerer & Fehr 2004; Henrich *et al.* 2001). As noted by Eckel & Grossman (1996) in their key paper, the behavior of subjects in dictator games is well documented and deviates from payoff maximization. Contrary to a strategy of maximizing fitness, participants do donate part of the money, with typical games resulting in around 80% of participants electing to donate some money and some 20% splitting the pie evenly (Forsythe *et al.* 1994). The common explanation given for these observed results is that participants are motivated by 'other-regarding preferences' (altruism or fairness) in addition to monetary payoffs.

Deviations from profit maximization as observed in economic game playing in general and the dictator game in particular underscore the paradox of human altruism (Fehr & Fischbacher 2003). Altruism is a phenomenon that has confounded evolutionary biologists because any evolutionary accounting of altruistic behavior would entail positive selection for behavioral genes that would seemingly reduce individual fitness. Nevertheless, human society is abundant with examples of prosocial or altruistic behavior.

Recognizing that many of the mechanisms underlying human altruistic behavior can be reproduced in the laboratory using economic game paradigms, brain science has recently focused on the anatomical, neurotransmitter and emotional substrates of facets of economic decision making. An integrated view of this emerging field of 'neuroeconomics' (Sanfey *et al.* 2006) has been generated by investigations from diverse disciplines including brain imaging (Fehr & Rockenbach 2004; de Quervain *et al.* 2004; Rilling *et al.* 2002; Singer *et al.* 2006; Stone *et al.* 2002), electrophysiology (Stuphorn 2006; van 't Wout *et al.* 2006), pharmacology (Kosfeld *et al.* 2005; Zak *et al.* 2005) and endocrinology(Takahashi 2004; Takahashi *et al.* 2006).

Although aspects of the neurobiological underpinnings of altruistic behavior are now being elucidated, a molecular genetic strategy toward exploring the contours of human altruism has been surprisingly lacking despite advances in understanding the role of specific genes in behavior (Ebstein 2006). Prosocial behavior is partially heritable (Rushton 2004; Scourfield *et al.* 2004), but only recently has a molecular genetic perspective on prosocial behavior been reported albeit using a self-report questionnaire (Bachner-Melman *et al.* 2005b).

An attractive candidate that we hypothesized might partially explain individual variance in altruistic giving in the dictator game is the *AVPR1a* gene. As we conjectured that allocation of funds in the dictator game may be modulated by trait-influenced patterns of social interactions, the AVPR1a receptor appeared a likely candidate for influencing pro-self vs. prosocial styles of behavior. In a previous study of this gene's role in psychopathology, we showed that deficits in social skills largely mediated association between *AVPR1a* repeat regions and autism (Yirmiya *et al.* 2006). We hypothesized that examining the relationship between *AVPR1a* repeat regions RS1 and RS3 (Thibonnier *et al.* 2000) and play in economic games would elucidate how this gene influences the social brain and particularly altruistic giving in healthy subjects. Additionally, it may serve to further our understanding of how *AVPR1a* mediates human behavioral disorders such as those observed in autism (Yirmiya *et al.* 2006) and schizophrenia. A relationship between *AVPR1a* promoter-region repeat length and individual differences in interpersonal behavior in *Homo sapiens* has not been previously shown.

An important focus of the current analysis was the length of the *AVPR1a* promoter-region repeat and its relationship to the sums allocated in the dictator game. Microsatellite length differences in the vicinity of the AVPR1a gene have been shown to be implicated in socially responsive behavioral differences in voles (Hammock & Young 2002, 2004, 2005; Hammock *et al.* 2005). Specifically, male prairie voles with long alleles, as compared with voles with short alleles, more quickly approach a novel social odor as well as unknown juvenile voles, indicating that the long alleles partially program for more 'trust-like' behavioral responses. Long-allele voles also display preferences for familiar partner females over an unknown sexually receptive female. Additionally, such voles invest more time in pup rearing, hinting at a more pro-kin behavioral style. In voles, the length effect of the *AVPR1a* repeat region on social behavior is mediated by transcriptional modulation and ensuing changes in anatomically specific protein concentrations. In humans, the role of promoter repeat region length in transcriptional regulation has yet to be addressed; this prompted us to compare variations in microsatellite length and *AVPR1a* messenger RNA (mRNA) expression levels in 15 post-mortem hippocampal samples.

Methods

Subjects

The participants in our ongoing studies of personality, primarily college students and their families, were recruited by word of mouth and advertisements on campus, as previously described (Bachner-Melman *et al.* 2005a,b). They were contacted by telephone and asked to participate in an online version of an economic game; 93% of contacted individuals agreed to participate. The sample consisted of 102 men and 101 women whose average age was 26.00 years (SD = 3.73). All subjects gave informed consent and the genetic study was approved by the local University and Hospital Internal Review Board and the Israeli Ministry of Health (Genetics Section). The subjects were initially recruited in two groups. The first group consisted of 142 individuals, and then 3 months later a second group of 66 participants were recruited.

All individuals that participated in the dictator game were also inventoried using the Bardi–Schwartz Universalism Value-expressive Behavior scale (Bardi & Schwartz 2003), that measures altruism and prosocial behavior. Parents did not participate in either the games or self-report questionnaires.

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Dictator game

The design used in Forsythe *et al.* (1994) was employed. The instructions to participants were:

> Dear participant,
> In this task you will be asked to make a decision which can earn you some money. The task will take place in pairs, in which case one of the participants will be Player A, and the other participant will be Player B. The assignment of Player A and Player B will be done randomly by the computer. You do not know the other participant and will not knowingly meet them in the future.
> In this task there are 50 points that Player A must decide how to distribute between himself and Player B. That is, Player A decides how many points he will keep for himself and how many points Player B will receive. For every point that he keeps for himself, Player A will receive 1₪ and for every point that he gives to Player 'B', Player 'B' will receive 1₪.
> This completes the task.
> Please press the button to continue.

Genotyping

The *AVPR1a* gene was genotyped as previously described (Bachner-Melman *et al.* 2005a). Figure 1 shows the location of the RS1 and RS3 promoter-region microsatellite repeats.

Measurement of AVPR1a mRNA levels in post-mortem hippocampal samples

To our knowledge, the current study is the first to measure *AVPR1a* mRNA levels using real-time polymerase chain reaction (PCR) in human brain.

Total RNA was extracted from 15 hippocampal post-mortem samples using a MasterPure™ DNA/RNA Purification kit (Epicentre) as described by the manufacturer. Post-mortem brain samples from 15 normal controls were obtained from the Rebecca L. Cooper Research Laboratories at the Mental Health Research Institute of Victoria, Australia (B.D.). Permission to carry out this study was obtained from the Ethics Committee of the Victorian Institute of Forensic Medicine and the North Western Mental Health Program Behavioral and Psychiatric Research and Ethics Committee. Complementary DNA (cDNA) was synthesized from 0.5–1 μg of RNA in a total volume of 20 μl using Reverse-iT 1st Stand Synthesis kit (ABgene, Surrey, UK). The amount of cDNA was quantified using real-time PCR (RotorGene 2000 real-time amplification system; Corbett Research, Eight Mile Plains, New South Wales, Australia). Phosphoglycerate kinase-1 (PGK-1) was the reference since it has been shown to be a stable housekeeping genes (Cheung *et al.* 2001; Seiler *et al.* 2004). Overall, similar results (data not shown) were obtained with a second housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (Bustin 2000). Amplification reactions were carried out in micro-capillary tubes using the following final concentrations: PGK-1: 100 nM each of the sense 5′: GCAGATTGTGTGGAATGGTC; anti-sense 5′: CCCTAGAAGTGGCTTTCACC. AVPR1a: 250 nM for each of the AVPR1A sense 5′-TCGGAAAACCCTACCATCACC-3′ and anti-sense 5′-TTGTTGGGCTTCGATTGTTAGAA–3′ primers. A 10 μl 1× ABsolute™ QPCR SYBR Green mix and 5 μl of cDNA were included in the reaction mixture. Cycling conditions for PGK-1 were as follows: denaturation (95°C for 15 min), amplification and quantification (95°C for 1 second, 60°C for 30 seconds and 72°C for 15 seconds). Cycling conditions for *AVPR1a* were as follows: denaturation (95°C for 15 min), amplification and quantification (95°C for 1 second, 62°C for 30 seconds and 72°C for 15 seconds). The 40 times repeated cycling was followed by a melting curve program (72–99°C with a heating rate of 1°C every 5 seconds and continuous fluorescence measurement).

The specificity of amplification was tested with a 3.5% agarose gel electrophoresis, and with real-time PCR melting analysis. Data were analyzed using RotorGene analysis software. The comparative CT method and the relative standard curve method were used for analysis. Equal amplification efficiencies (approximately 0.99) were confirmed for target and reference genes. To correct for variations of RNA amounts and cDNA synthesis efficacy, primers for the detection of *PGK-1* (housekeeping gene) were generated.

The PCR primers for *AVPR1a* were obtained from PrimerBank (Wang & Seed 2003) http://pga.mgh.harvard.edu/primerbank/index.html. The amplicon size was 213 bp (primer ID 4502331a3). Amplicon locations were forward: 973–993 nt and back: 1186–1164 nt.

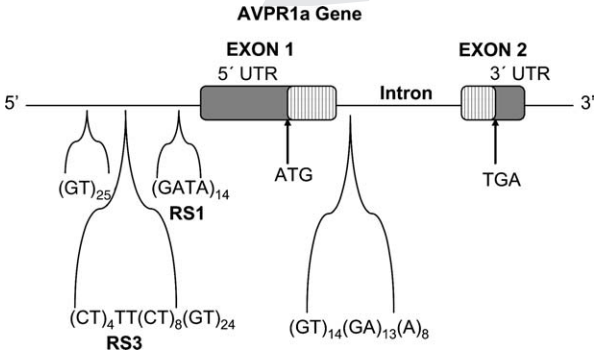

Figure 1: Location of AVPR1a microsatellite repeats (Thibonnier *et al.* 2000). The first codon is represented by ATG.

Genotyping of post-mortem brain samples

The DNA was extracted from 5 μg of post-mortem cerebellum samples (corresponding to the same hippocampal samples) using MasterPure™ DNA Purificaton kit (Epicentre) as described by the manufacturer. Amplification of the RS3 vasopressin was achieved using the following pair of primers as previously described in previous investigations from our laboratory (Bachner-Melman *et al.* 2004, 2005a; Yirmiya *et al.* 2006): forward: (fluorescent) 5′-CCT GTA GAG ATG TAA GTG CT-3′; reverse: 5′-TCT GGA AGA GAC TTA GAT GG-3′.

Reaction mixture contained 0.5 μM of each primer. A RedyMix master mix was used (ABgene, Surrey, UK) at a magnesium concentration of 1.5–2.5 mM $MgCl_2$.

Cycling conditions for were as follows: denaturation (95°C for 30 seconds), 30 cycles of amplification (95°C for 30 seconds, 55°C for 30 seconds and 72°C for 40 seconds) and a final extension step of 72 °C for 10 min. The PCR product was analyzed on an ABI 310 DNA Analyzer.

Statistical analysis

We used the logistic-based variant of the transmission disequilibrium test (TDT) so-called ETDT (Sham & Curtis 1995) to assess association (and linkage) without the confounding effect of population stratification. The various tests are implemented in the latest version of the program UNPHASED version 3 (http://www.rfcgr.mrc.ac.uk/fdudbrid/software/unphased/), which is freely downloaded from the author's site. UNPHASED (Dudbridge 2003) is a suite of programs for association analysis of multilocus haplotypes from unphased genotype data.

For single-locus and haplotype analysis, UNPHASED calculates overall global *P* values that consider multiple testing of haplotypes. Those values are included in all of the tables (Global *P* values). However, regarding the more complicated problem of how to correct for multiple testing in association studies when there are potentially approximately 30 000 genes in the human genome, the reader is referred to the insightful article by Neale and Sham (2004) that discusses this problem. In the current study, the *P* values are nominal and not corrected for multiple testing. Simple Bonferroni correction is not justified because the two microsatellites are in moderate linkage disequilibrium.

13 In addition to UNPHASED we also used PBAT-HELIXTREE (Lange *et al.* 2004; Steen & Lange 2005) (http://www.goldenhelix.com/helixtree_pbat.html), which permits multivariate analysis of family-based association tests (Lange *et al.* 2003; Randolph *et al.* 2005) that can be used with multiple phenotypes and multiple genes. In the current study, we used the PBAT multivariate FBAT-PC statistic to examine simultaneously two related phenotypes in the self-report questionnaires that measure facets of altruism, the Bardi–Schwartz Benevolence and Universalism Value-expressive Behavior scales (Bardi & Schwartz 2003). PBAT is designed for single nucleotide polymorphisms (biallelic polymorphisms) and we therefore analyzed the AVPR1a RS3 repeat region by employing the short and long classification grouping.

All other statistical tests were carried out using SPSS version 13 (Windows).

Results

Dictator game

Figure 2 shows the allocation of shekels (₪), out of a 50 ₪ or $12 pie, given to the recipient by the 'dictators' in this study. 14.9% of the participants allocated nothing to the 'other', 34.6% allocated half their endowment (25 ₪) and 6.7% allocated the entire sum. No gender differences in allocation sums were observed. Table 1 presents the individual dictator allocation amounts grouped by RS3 allele repeat length and the average allocations per individual allele length. As shown in Table 1, the allele repeat distribution, similar to other short-tandem repeats, is characterized by relatively rare alleles at the extremes of the distribution. For example, the bin of 310–319 bp alleles is represented by only five subjects. This characteristic distribution of low-frequency alleles in microsatellite repeats has prompted numerous investigators to group repeat lengths into short vs. long bins in genetic analyses (Hietala *et al.* 2007; Leibowitz *et al.* 2006; Lowe *et al.* 2000; Morgan *et al.* 2005; Terry *et al.* 2005; Ullrich *et al.* 2005). We chose the short and long lengths of the RS3 and promoter length so that approximately equal numbers of participants were present in each group. Any other split led to very small groups in either the long or short category for a total sample size of $n = 203$.

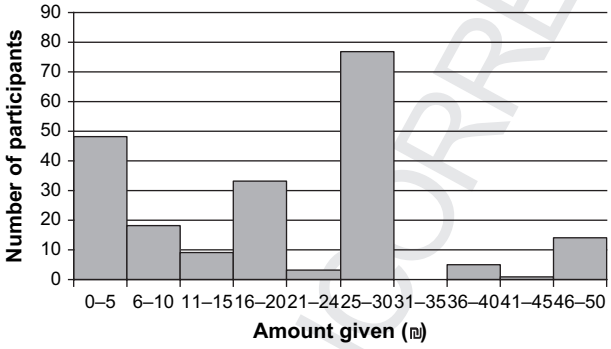

Figure 2: Distribution of allocation sums by participants in dictator game. 25 ₪ (shekels) was the modal value in this distribution and was used as the cutoff point to divide participants into low and high allocators. Altogether, 46% of the participants were designated as high allocators. The current results can be compared with Forsythe *et al.* (1994) who explored the replication and statistical properties of the dictator game. The standard perfect equilibrium analysis of the dictator game begins with the assumption that each player prefers more money to less (Bolton & Ockenfels 2000). In the dictator game, the so-called 'Dictator' should keep all the money. However, in the case of the $10 game, fully 79% left a positive amount of money, with 20% leaving half. The mode of the distribution was $3 or 30%. Notably, the study by Forsythe *et al.* (1994) showed distributions of dictator giving, which are both anomalous to standard economic theory of maximizing profit as well as robustly replicable.

Population-based analysis: long/short classification scheme

As the *AVPR1a* promoter repeat length in the vole is the major determinant of this gene's transcription pattern in the brain and ensuing social behavior (Hammock & Young 2005), we examined the relationship between repeat length and allocation.

As shown in Fig. 3, significantly fewer participants with short versions of the *AVPR1a* RS3 repeat allocated high sums to the 'other' than participants with long versions (SPSS Crosstabs two-tailed: likelihood ratio = 14.75, $P = 0.001$, df = 2). Additionally, the presence of RS3 long repeats had an additive effect on allocation amounts. A bivariate (sex × RS3 genotype) ANOVA found a main effect for RS3 genotype, $F(2, 191) = 4.29$, $P = 0.015$; no main effect for sex, $F(1, 191) = 0.18$, NS; and no interaction between sex and RS3 length, $F(2, 191) = 0.13$, NS, in allocation sums. SPSS *post hoc* analysis using the Tukey's HSD test showed a significant difference between short/short vs. long/long ($p = 0.025$). Subjects homozygous for short repeats gave 15.4 ₪ (males, 13.8; females, 15.7), whereas subjects homozygous for long repeats gave 22.2 ₪ (males, 22.6; females, 22.0), an effect size of approximately 0.5 SD (Fig. 4).

To test the robustness of the analysis, we tested two additional cutoffs (308–327 bp and 329–343 bp; 308–323 bp and 325–343 bp). Again, there is a trend that subjects with short RS3 alleles allocate lower sums than subjects with the long alleles. In the second cutoff (Fig. 3b), 40% of short/short subjects allocated 25 ₪ or more, whereas 55% of long/long subjects allocated 25 ₪ or more. In the third cutoff (Fig. 3c), 25% of short/short subjects allocated 25 ₪ or more, whereas among long/long subjects 51% allocated 25 ₪ or more. However, the results do not attain significance at the $P = 0.05$ level in 3b and 3c, likely because of the lower power when relying on smaller groups to estimate the effect (long/long: $n = 18$, Fig. 3b; short/short: $n = 8$, Fig. 3c).

Family-based analysis

We also examined the robustness of our first analysis by using UNPHASED, that implements a family-based design and avoids the conundrum of population admixture or stratification, and tested association between money allocations and RS1 and RS3 repeats. Table 2 presents the results for testing each individual RS3 repeat allele for association with dictator game allocations. *P* values for both individual alleles as well as a global *P* value that corrects for multiple testing of individual alleles in repeats are calculated by UNPHASED. As expected from our first analysis, significant association and dictator

Table 1: Allocation sums in the dictator game grouped by individual RS3 allele length

	AVPR1A RS3 Repeat length (base pairs)														
Allocated	310	312	317	319	321	323	325	327	329	331	333	335	337	339	341
0	1	0	0	0	2	13	17	7	9	3	2	0	5	2	1
1	0	0	0	0	1	1	6	2	0	1	0	0	0	1	0
2	0	0	0	0	0	1	2	2	2	1	0	0	0	0	0
3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
5	0	0	0	0	0	1	6	2	0	2	0	0	1	0	0
10	0	0	0	1	2	2	11	7	4	4	0	0	0	1	0
15	0	0	0	0	1	3	11	1	0	1	1	0	0	0	0
18	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
20	0	0	1	0	1	13	21	15	5	6	0	0	1	1	0
22	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
23	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0
25	1	1	0	0	3	14	36	33	20	11	0	2	13	4	0
30	0	0	0	0	1	0	5	2	1	0	1	0	0	0	0
40	0	0	0	0	1	2	3	1	1	0	2	0	0	0	0
45	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
50	0	0	0	0	2	3	3	10	6	2	0	0	2	0	0
Count	2	1	1	1	14	54	125	87	49	31	6	3	22	9	1
Frequency	0.0049	0.0025	0.0025	0.0025	0.0345	0.1330	0.3079	0.2143	0.1207	0.0764	0.0148	0.0074	0.0542	0.0222	0.0025
Average	12.50	25.00	20.00	10.00	21.50	16.96	17.11	22.55	21.14	18.16	20.83	24.33	20.45	14.56	0

game allocations was significant (global $P < 0.05$) only for the RS3 repeat (entire sample: global P value: likelihood ratio $\chi^2 = 11.73$, df $= 4$, global P value $= 0.019$). The third most common allele (12%), 329 bp, showed significant association with allocation ($P = 0.008$).

Split-sample design

We also separately examined significance in the initial and replication samples that we recruited. In the split-sample design, two groups of subjects (an initial sample and a replication sample) were analyzed independently; allowing us to examine the replicability of the initial study findings.

As shown in Table 2, in the first group of subjects recruited (initial sample: $n = 142$ participants, 70% total sample) we observed significant family-based association (global P value: likelihood ratio $\chi^2 = 17.16$, df $= 5$, $P = 0.004$). Following these initial positive findings, a second replication sample was recruited and similar results were observed (replication sample: $n = 66$, 30%) (global P value: likelihood ratio $\chi^2 = 15.09$, df $= 4$, $P = 0.005$).

Similarly, in the population-based analysis, when we examined the relationship between repeat length (short/long) and allocation (high/low), significant results were observed in both the initial sample and the replication sample (initial sample: likelihood ratio $= 11.85$, $P = 0.001$, df $= 1$; replication sample: likelihood ratio $= 4.23$, $P = 0.04$, df $= 1$).

Self-report measure of altruism

Participants reported their own prosocial behavior with the Value-expressive Behavior scale by Bardi and Schwartz (2003). Two subscales were used, that represent two different aspects of prosocial values. The universalism behavior subscale taps behaviors that represent a prosocial motivation for understanding, appreciation, tolerance and protection of the welfare of all people (e.g. 'Make sure everyone I know receives equal treatment'; 'Donate money for saving people who suffer from war, famine, etc. in distant countries'). Significant association was observed between scores on the Universalistic behavior subscale and the RS3 repeat (Table 3).

The other subscale is the benevolent behavior subscale (Bardi & Schwartz 2003). This subscale taps behaviors that represent a prosocial motivation to help and support others with whom one is in close or daily social contact (e.g. 'agree easily to lend things to neighbors'; 'help my friend to perform tasks such as moving and studying'). Significant association was observed between scores on the benevolent behavior subscale and the RS3 repeat (Table 3).

Relation between AVPR1a promoter-region repeat length and hippocampal mRNA levels

In humans, the role of the *AVPR1a* promoter repeats on this gene's transcription pattern has not been studied, prompting us to examine the relationship between *AVPR1a* RS3 repeat length (grouped into long/long, long/short and short/short genotypes) and *AVPR1a* mRNA levels in 15 post-mortem hippocampal samples (Fig. 5). As a significant correlation was observed between subject age and *AVPR1a* mRNA levels ($r = 0.604$, $P = 0.017$, $n = 15$), the standardized residuals (mRNA levels regressed on age) were used as the independent variable in the ANOVA analysis. Long *AVPR1a* RS3 repeats were associated with higher *AVPR1a* hippocampal mRNA levels than short repeats (One-way ANOVA: $F = 15.04$, $P = 0.001$, df $= 14$) suggesting a molecular genetic functional basis for the observation that participants with the long RS3 repeats allocate more money than participants with the short repeats. Tukey's HSD test showed significant *post hoc*

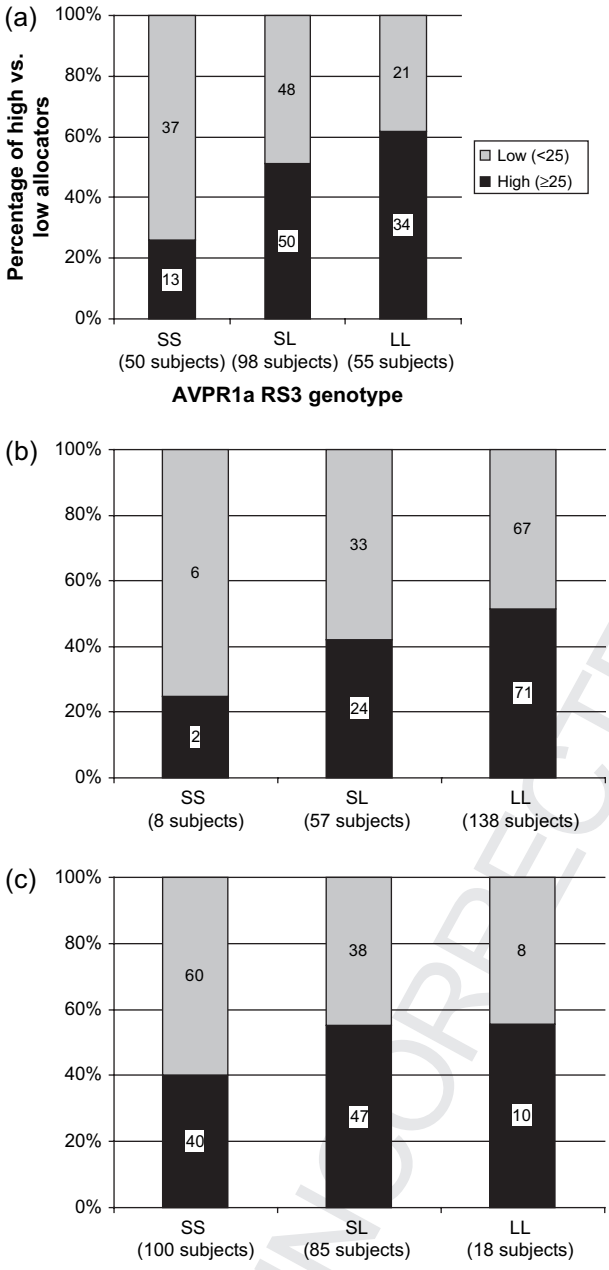

Figure 3: Mode of giving in the dictator game. High vs. low allocation amounts in ₪ (shekels) grouped by short (308–325 bp) vs. long (327–343 bp) *AVPR1a* RS3 promoter-region repeat length (SPSS v13 Crosstabs two-tailed: likelihood ratio = 14.75, $P = 0.0006$, df = 2). Percentages indicate ratio of high (≥25 ₪) vs. low (<25 ₪) allocators for each of the three genotype groupings. The allele frequencies for RS1 and RS3 are shown in Fig. 5. Figure 3b,c show a similar display, however with alternative short/long groupings: 3b, short (308–327 bp), long (329–343 bp); 3c, short (308–323 bp), long (325–343 bp).

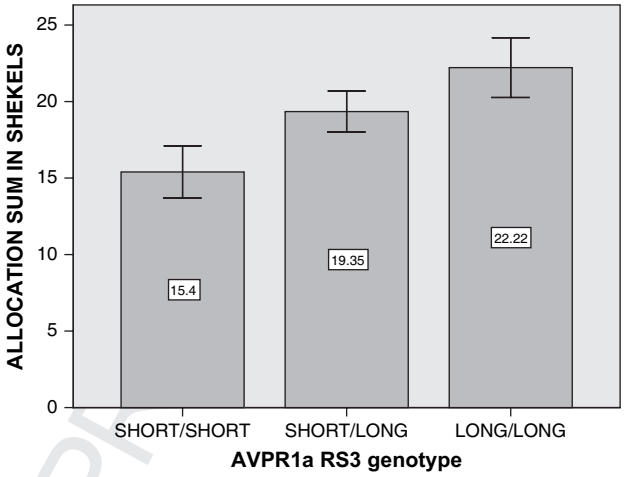

Figure 4: Allocation amount (continuous variable) grouped by AVPR1a RS3 long vs. short genotype. Error bars are ± SEM. One-way ANOVA: $n = 203$, $F = 3.456$, $P = 0.033$. SPSS *post hoc* analysis using the Tukey's HSD test showed a significant difference between short/short vs. long/long ($P = 0.025$).

effects between short/long vs. long/long ($P = 0.0004$) and short/short vs. long/long ($P = 0.003$). When the genotypes were collapsed into short vs. long alleles, the long alleles were associated with higher AVPR1a mRNA levels ($F = 70.22$, $P = 0.0004$, df = 29).

Discussion

We provide the first evidence that DNA polymorphisms, represented by promoter-region repeat length, are responsible for individual differences in human altruism as assessed in donation of money. The *AVPR1a* promoter-region repeat regions, in particular the length of this region, were associated with the amounts allocated in the dictator game. In both animal and human studies, this gene has been shown to contribute to several facets of social recognition, social memory and their attendant behaviors (Hammock & Young 2002, 2005; Hammock *et al.* 2005). Hammock *et al.* (2005) have shown, by analyzing brain regions from 20 adult male voles genotyped for the promoter-region repeat region, that AVPR1a binding variation was strongly correlated with microsatellite genotype in three main brain regions: the main and accessory olfactory bulbs, the amygdala and the thalamus. Most importantly, AVPR1a levels in those and other brain regions correlate with anxiety-related and social behaviors. Remarkably, parallel results were obtained in the present study in another species, *Homo sapiens*, separated by millions of years of evolutionary time from the vole. We showed a significant relationship between *AVPR1a* promoter-region repeat length and altruistic giving involving real money payoffs, and secondly, that repeat length determines transcription of this gene in human post-mortem hippocampal samples. Notably, association was also shown between the *AVPR1a* RS3 microsatellite in a robust family-based genetic analysis using a split-sample design.

Table 2: Single-locus analysis of AVPR1a RS3 repeat lengths and dictator allocations

Allele	Count	MarFreq	AddVal	χ^2	*P* value	Common
Entire sample						
323	98	0.128	1.1600	5.4320	**0.0198**	+
325	223	0.290	1.1950	0.0143	0.9050	+
327	171	0.223	1.2090	0.6843	0.4081	+
329	95	0.124	1.2320	6.9530	**0.0084**	+
331	66	0.086	1.1960	0.4963	0.4811	+
Initial sample						
323	69	0.130	56.190	7.470	**0.00627**	+
325	154	0.290	56.240	0.014	0.90680	+
327	117	0.220	56.250	0.062	0.80400	+
329	64	0.120	56.310	10.160	**0.00143**	+
331	46	0.086	56.280	0.392	0.53140	+
337	30	0.056	56.280	0.079	0.77900	+
Replication sample						
323	29	0.12	234.4	2.432	0.11890	+
325	69	0.29	234.4	0.509	0.47570	+
327	54	0.23	234.4	15.610	**0.00008**	+
329	31	0.13	234.4	6.546	**0.01051**	+
331	20	0.08	234.3	6.528	**0.01062**	+

MarFreq is the marker frequency in the population. AddVal gives the additive change in mean for a haplotype, relative to the reference haplotype. So if the reference allele has mean *x*, a relative mean of *y* means that the allele has mean $x + y$. More precisely, if one chromosome is chosen at random in the population, the relative mean refers to the expected trait value of the subject carrying that chromosome, given its allele distribution. Only alleles with frequencies $\geq$0.05 were used in the calculation of the global *P* value. In the quantitative trait locus option, we used the 'Model Normal Distribution' option.

Although the main aim of the current investigation was to examine the role of the *AVPR1a* gene in explaining individual differences in altruism using an economic game paradigm that involves real money payoffs, we also examined association between the *AVPR1a* promoter-region repeats and scores on two self-report scales that also reflect facets of human altruism. The first scale we employed was the Universalism subscale of the Value-expressive Behavior scale by Bardi & Schwartz (2003). Universalism includes understanding, appreciation, tolerance and protection of the welfare of all people and of nature (broadminded, wisdom, social justice, equality, a world at peace, a world of beauty, unity with nature, protecting the environment). The second scale is the Benevolence subscale of the Bardi–Schwartz scale, that refers to the preservation and enhancement of the welfare of people with whom one is in frequent personal contact (friendship, help and loyalty). Significant associations were observed between the promoter-region *AVPR1a* RS3 repeat and both subscales. Thus, the *AVPR1a* gene not only contributes to individual differences in money allocations in the dictator game but the long alleles of the promoter RS3 repeat region are associated with higher scores on two self-report measures of human altruism.

It is possible that using the mode as the cutoff for the short–long classification of RS3 microsatellite length may be considered arbitrary and the significant results we report could be the result of type I error. We used a cutoff at the mode, which allowed for a balanced distribution of subjects in each genotype category, rather than the small group sizes obtained when using the alternative cutoffs. Effects using the two additional cutoffs were not significant, but displayed a positive trend in the same direction, calling for replication of our results in an independently recruited sample to increase confidence in the findings. The use of the mode is further supported by our transcriptional study that shows the short/short genotype correlates with less AVPR1a mRNA.

In the past decade, numerous observations have suggested that repeat regions such as the *AVPR1a* RS3 dinucleotide microsatellite investigated in the current study participate in the regulation of gene expression. Interestingly, repeat regions have evolved more rapidly in humans than in chimpanzees and other primates, resulting overall in longer and more polymorphic repeats in humans, especially dinucleotides (Rubinsztein *et al.* 1995a,b). Many repeat regions affect gene expression often as regulatory sequences that serve as transcription factor-binding sites (Kashi *et al.* 1997). Association between promoter-region repeats and gene transcription is a common observation for many human microsatellite polymorphisms (Hietala *et al.* 2007; Morgan *et al.* 2005) and an increasing number of intronic short tandem repeat have been found to modulate transcription processes by their effect on secondary DNA structure or other unknown mechanisms. Short tandem repeats also act as regulatory

Table 3: Association between *AVPR1*a RS3 and Universalism and Benevolence

Phenotype	Marker	Allele	#FAM	*P* value (FBAT)	Direction of effect
Benevolent behavior	RS3LS	Long	34	0.388	+
	RS3LS	Short	36	0.033	−
Universalistic behavior	RS3LS	Long	34	0.239	+
	RS3LS	Short	36	0.044	−
Both prosocial behaviors (multivariate)	RS3LS	FBAT-PC statistic	80	0.035	+

Universalism and Benevolence are from the Bardi–Schwartz Universalism Value-expressive Behavior scale (Bardi & Schwartz, 2003). #FAM, the number of informative families (after collapsing repeat regions to long/short lengths). FBAT-PC χ^2 is the multivariate statistic. Direction of effect, the direction of association (+ is a positive section; −is a negative association).

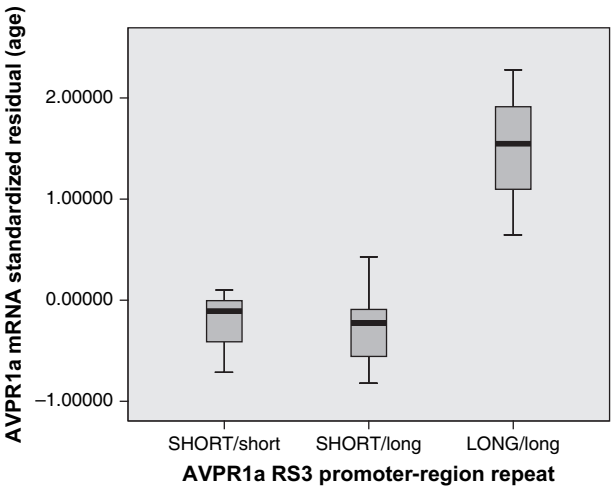

Figure 5: Hippocampal *AVPR1a* mRNA levels grouped by *AVPR1a* RS3 promoter repeat by genotype length. The data are presented as a SPSS box plot. Control subjects had no contact with any psychiatric service prior to death, had not received antipsychotic medication, had not died by suicide or had any neurological disorder. Age: 46.6 years ± 15.2 (SD); sex: 4 females/11 males, post-mortem interval: 42.0 h ± 16.4 (SD); and pH of brain tissue: 6.3 ± 0.22 (SD). Post-mortem mRNA levels correlated only with age. We also analyzed the data using the non-parametric Kruskal–Wallis statistic ($\chi^2 = 6.8$, 2 df, $P = 0.033$).

factors in introns and untranslated region (UTR) regions (Riley & Krieger 2005). One of the clearest examples is the polymorphic pentanucleotide repeat located within the 5′-UTR of the p53-induced gene *PIG3*. The repeat is necessary and sufficient for transcriptional activation of *PIG3* by p53. Higher number of repeats is correlated with higher transcriptional activation by p53 (Contente *et al.* 2002). Another example is the androgen receptor polyglutamine CAG repeat in exon 1 of the gene whose length is inversely associated with transcriptional activity (Westberg *et al.* 2001), androgen levels (Shimbo *et al.* 2005) as well as diverse phenotypes such as cognition in older men (Yaffe *et al.* 2003) and cardiovascular disease (Alevizaki *et al.* 2003).

The results presented in the current investigation suggest that the *AVPR1a* RS3 promoter repeat serves a similar regulatory function in humans because we observed an association between repeat length and mRNA in post-mortem hippocampal samples. Nevertheless, the possibility that RS3 is in linkage disequilibrium with other functional polymorphisms in the 5′ upstream region, that might explain some of our results, cannot be excluded.

Game theory and its derivatives such as behavioral economics and neuroeconomics are not only important in explaining modern day economic behavior but also are important in evolutionary psychology and particularly the evolution and maintenance of high levels of altruism, an inherent human trait distinguishing us from all other vertebrate species. The mechanisms by which human altruism evolved remain controversial despite the overwhelming evidence for its widespread occurrence in both dyadic and large social group interactions (Fehr & Rockenbach 2004). Interestingly, a recent study (Bowles 2006) presents a theoretical model for evolution of human altruism and suggests that genetic differences between early human groups are likely to have been great enough so that lethal intergroup competition could account for the evolution of altruism. As noted by Bowles (2006), nothing in his study suggests that a genetic predisposition favoring human altruism exists or that cultural or other possible explanations of human altruism are of lesser importance. However, the current investigation [and our previous investigation using self-report measures of altruism (Ebstein 2006)] nevertheless strengthens the notion that genes partially contribute to voluntary actions that promote the interest of others, for reasons other than self-interest. Our evidence suggests that humans are partially hard-wired for altruistic allocations of money in an economic game and imply that similar genetic determinants are important outside the economic game's laboratory. These determinants are represented by individual, allelic differences across individuals, the grist for the evolutionary mill.

Although the powerful tools of experimental economics and brain imaging have been used to partially explain the proximate mechanisms of human altruism (Fehr & Rockenbach 2004; de Quervain *et al.* 2004; Rilling *et al.* 2002, 2004), a molecular genetics strategy has yet to be employed toward further understanding the source of individual differences in pro-self vs. prosocial styles of playing economic games. Importantly, although experimental economics and imaging studies can inform regarding which proximate strategies people employ in economic game playing, they cannot distinguish between state vs. trait biases in human behavior. Additionally, there is strong evidence that prosocial behavior in real life is moderately heritable (Knafo & Plomin 2006). The current report extends the classical twin approach and is the first investigation to examine the role of a specific genetic polymorphism in contributing to individual differences in how people play economic games.

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Insert 'inferior' character	(As above)	over character e.g.
Insert full stop	(As above)	
Insert comma	(As above)	
Insert single quotation marks	(As above)	or and/or or
Insert double quotation marks	(As above)	or and/or or
Insert hyphen	(As above)	
Start new paragraph		
No new paragraph		
Transpose		
Close up	linking characters	
Insert or substitute space between characters or words	through character or where required	
Reduce space between characters or words	between characters or words affected	