

# האוניברסיטה העברית בירושלים

## THE HEBREW UNIVERSITY OF JERUSALEM

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**INDIVIDUAL DIFFERENCES IN ALLOCATION OF FUNDS IN THE DICTATOR GAME ASSOCIATED WITH LENGTH OF THE ARGININE VASOPRESSIN 1a RECEPTOR (AVPR1a) RS3 PROMOTER-REGION AND CORRELATION BETWEEN RS3 LENGTH AND HIPPOCAMPAL mRNA**

by

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Individual Differences in Allocation of Funds in the Dictator Game Associated with Length of the Arginine Vasopressin 1a Receptor (*AVPR1a*) RS3 Promoter-region and Correlation between RS3 Length and Hippocampal mRNA

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## Abstract

Human altruism is a widespread phenomenon that puzzled evolutionary biologists since Darwin. Economic games illustrate human altruism by demonstrating that behavior deviates from economic predictions of profit maximization. A game that most plainly demonstrates 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 & RS3 promoter-region repeat polymorphisms. Parents did not participate in online game playing.

Since 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-sq = 11.73,  $DF= 4$ ,  $p$ -value = 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 postmortem hippocampal mRNA levels than short RS3 repeats (One way-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.

## Introduction

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 demonstrates 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 and Grossman in their key paper (Eckel & Grossman, 1996) 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 (De Quervain *et al.*, 2004, Fehr & Rockenbach, 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 towards exploring the contours of human altruism has been surprisingly lacking despite advances in understanding the role of specific genes in behavior (Ebstein, 2006a). 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. Since 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 versus pro-social styles of behavior. In a previous study of this gene's role in

psychopathology, we demonstrated 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 demonstrated.

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 *et al.*, 2005, Hammock & Young, 2002, Hammock & Young, 2004, Hammock & Young, 2005). Specifically, male prairie voles with long alleles, as compared to 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* mRNA expression levels in 15 postmortem 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, Bachner-Melman *et al.*, 2005b). They were contacted by telephone and asked to participate in an online version 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 (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 three 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.

### *Dictator Game*

The design used in Forsythe, Horowitz, Savin, and Sefton (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 PCR in human brain.

Total RNA was extracted from 15 hippocampal postmortem samples using a MasterPure™ DNA/RNA Purification kit (Epicentre) as described by the manufacturer. Postmortem brain samples from 15 normal controls were obtained from the Rebecca L. Cooper Research Laboratories at the Mental Health Research Institute of Victoria, Australia (Brian Dean). 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. 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 House, Blenheim Road, 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). 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 (GAPDH) (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; antisense 5': CCCTAGAAGTGGCTTTCACC. **AVPR1a**: 250nM for each of the AVPR1A sense 5'TCGGAAAACCCTACCATCACC 3' and antisense 5' TTGTTGGGCTTCGATTGTTAGAA 3' primers. 10µl 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 quantitation (95°C for 1 sec, 60°C for 30 sec and 72°C for 15 sec). Cycling conditions for *AVPR1a* were as follows: denaturation (95°C for 15 min), amplification and quantitation (95°C for 1sec, 62°C for 30 sec and 72°C for 15 sec).The 40 times repeated cycling was followed by a melting curve program (72-99°C with a heating rate of 1°C every 5 sec 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 (~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 phosphoglycerate kinase-1 (*PGK-1*) (housekeeping gene) were generated.

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.

### **Genotyping of postmortem brain samples**

DNA was extracted from 5µg of postmortem 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.*, 2005a, Bachner-Melman *et al.*, 2004, 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  $\mu$ M of each primer. A RedyMix master mix was used (Abgene, Surrey, UK) at a magnesium concentration of 1.5-2.5mM MgCl<sub>2</sub>.

Cycling conditions for were as follows: denaturation (95°C for 30 sec), 30 cycles of amplification (95°C for 30 sec, 55°C for 30 sec and 72°C for 40 sec) and a final extention 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 that discusses this problem (Neale & Sham, 2004). In the current study, the *p*-

values are nominal and not corrected for multiple testing. Simple Bonferroni correction is not justified since the two microsatellites are in moderate linkage disequilibrium.

In addition to UNPHASED we also used PBAT-HelixTree (Lange *et al.*, 2004, Steen & Lange, 2005) ([http://www.goldenhelix.com/helixtree\\_pbat.html](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 & Universalism Value-expressive behavior scales (Bardi & Schwartz, 2003). PBAT is designed for SNPs (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

### A. 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 5 subjects. This characteristic distribution of low frequency alleles in microsatellite repeats has prompted numerous investigators to group repeat lengths into short versus 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.

#### *Population-based analysis: long/short classification scheme*

Since 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 Figure 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 X RS3 genotype) analysis of variance found a main effect for RS3 genotype,  $F(2, 191)=4.29$ ,  $P=0.015$ , no main effect for sex,  $F(1, 191)=0.18$ , n.s., and no interaction between sex and RS3 length,  $F(2, 191)=0.13$ , n.s., in allocation sums. SPSS post-hoc analysis using the Tukey HSD test showed a significant difference between short/short versus 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 (Figure 4).

To test the robustness of the analysis, we tested two additional cutoffs (308-327 bp & 329-343 bp; 308-323 & 325-343). 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 shekels or more whereas 55% of long/long subjects allocated 25 shekels or more. In the third cutoff (Fig 3c) 25% of short/short subjects allocated 25 shekels or more whereas among long/long subjects 51% allocated 25 shekels or more. However, the results do not attain significance at the  $p=0.05$  level in 3b and 3c, likely due to 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 Game allocations was significant (global  $p < 0.05$ ) only for the RS3 repeat (entire sample: global p value: Likelihood ratio chi-sq = 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-sq = 17.16, DF = 5, p-value = 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-sq = 15.09, DF = 4, p-value = 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 (Bardi & 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).

#### B. 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 postmortem hippocampal samples (Figure 5). Since 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 HSD test showed significant post-hoc effects between short/long versus long/long ( $p=0.0004$ ) and short/short versus long/long ( $p=0.003$ ). When the genotypes were collapsed into short versus 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 *et al.*, 2005, Hammock & Young, 2002, Hammock & Young, 2005). Hammock and her colleagues (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 demonstrated 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 postmortem hippocampal samples. Notably, association was also demonstrated between the AVPR1a RS3 microsatellite in a robust family-based genetic analysis using a split-sample design.

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 and Schwartz (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, 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 due to 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, Rubinsztein *et al.*, 1995b). 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 factors in introns and 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 are 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, since we observed an association between repeat length and mRNA in postmortem 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 (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, 2006b) 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 (De Quervain *et al.*, 2004, Fehr & Rockenbach, 2004, Rilling *et al.*, 2002, Rilling *et al.*, 2004) a molecular genetics strategy has yet to be employed towards further understanding the source of individual differences in pro-self versus pro-social 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 versus 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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Table 1. Allocation sums in the Dictator Game grouped by individual RS3 allele length



<div style="text-align: center;">                        Allocated                 </div>	AVPRIA RS3 REPEAT LENGTH (base pairs)														
	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

Table 2. Single locus analysis of AVPR1a RS3 repeat lengths and Dictator allocations

Allele	Count	MarFreq	AddVal	Chi-sq	P-value	Common
Entire sample						
323	98	0.128	1.1600	5.4320	<b>0.0198</b>	+
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	<b>0.0084</b>	+
331	66	0.086	1.1960	0.4963	0.4811	+
Initial sample						
323	69	0.130	56.190	7.470	<b>0.00627</b>	+
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	<b>0.00143</b>	+
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	<b>0.00008</b>	+
329	31	0.13	234.4	6.546	<b>0.01051</b>	+
331	20	0.08	234.3	6.528	<b>0.01062</b>	+

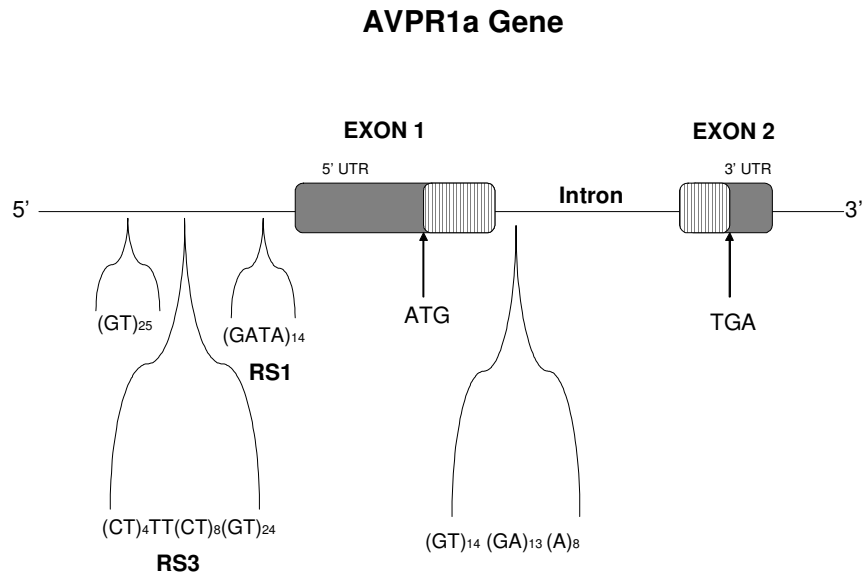
MarFreq is the marker frequency in the population. AddVal gives the additive change in mean for a haplotype, relative to the reference haplotype. So if the reference allele has mean  $x$ , a relative mean of  $y$  means that the allele has mean  $x+y$ . More precisely, if one chromosome is chosen at 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 QTL option we used the 'Model Normal Distribution' option.

Table 3. Association between *AVPR1a* RS3 and Universalism and Benevolence

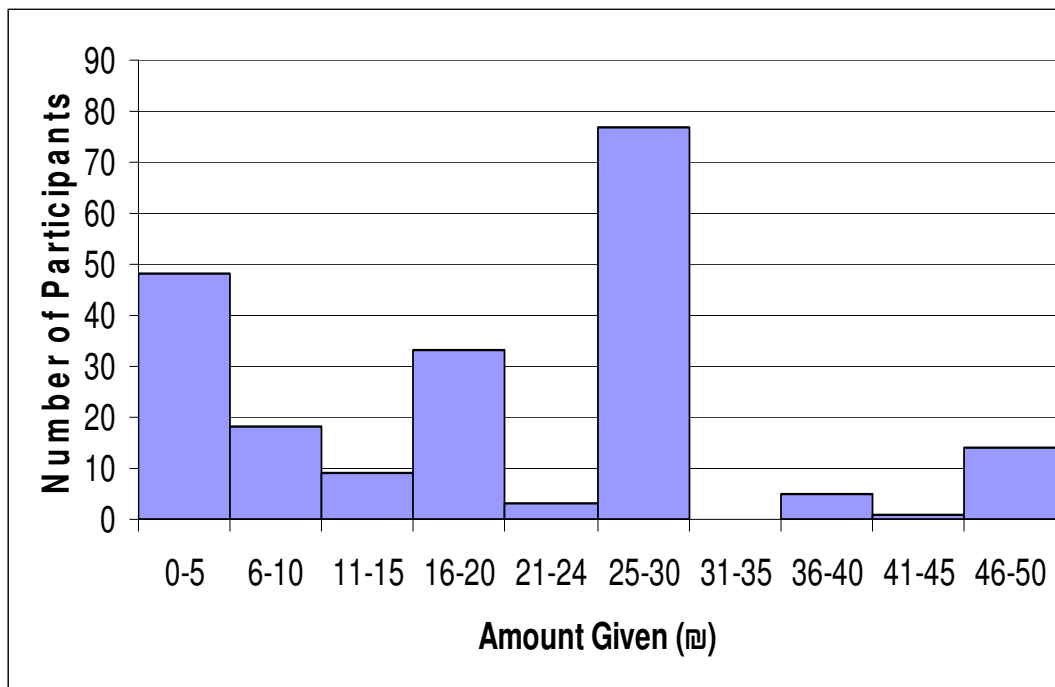
Phenotype	Marker	Allele	#FAM	P value (FBAT)	Direction of effect
Benevolent behavior	RS3LS	Long	34	0.388	+
	RS3LS	Short	36	0.033	-
Universalistic behavior	RS3LS	Long	34	0.239	+
	RS3LS	Short	36	.044	-
Both prosocial behaviors (multivariate)	RS3LS	FBAT-PC statistic	80	0.035	+

Universalism and Benevolence are from the Bardi-Schwartz Universalism Value-expressive Behavior scale (Bardi & Schwartz, 2003). #FAM is the number of informative families (after collapsing repeat regions to long/short lengths). FBAT-PC chi-sq is the multivariate statistic. Direction: the last column in the table indicates the direction of association (+ is a positive section; - is a negative association).

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



**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 to Forsythe et al (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 (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.



**Figure 3.** Mode of giving in the Dictator Game. High versus low allocation amounts in ₪ (shekels) grouped by short (308-325 bp) versus 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 ( $\geq 25$  ₪) vs. low ( $< 25$  ₪) allocators for each of the three genotype groupings. The allele frequencies for RS1 and RS3 are shown in Figure 5. Figures 3b and 3c 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 3.

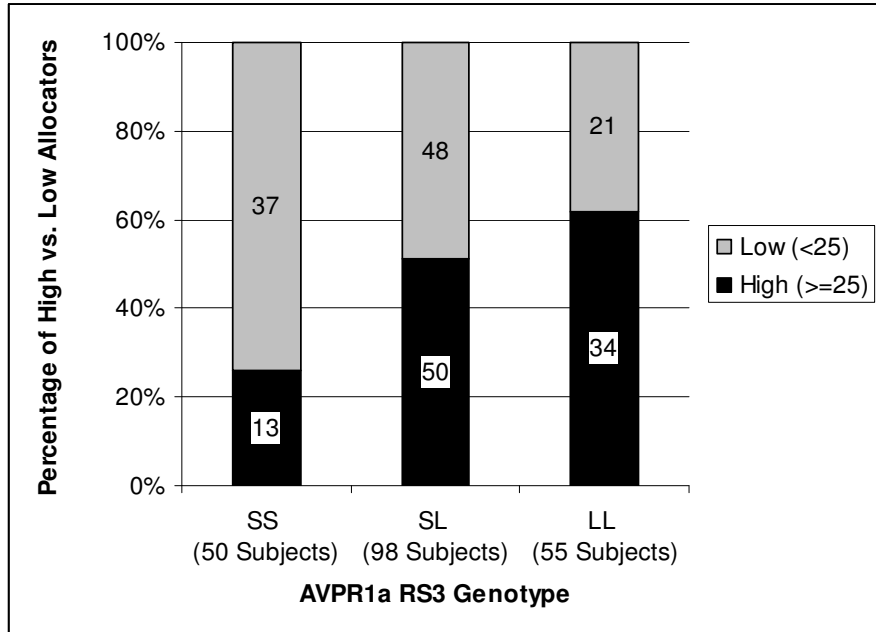


Figure 3b.

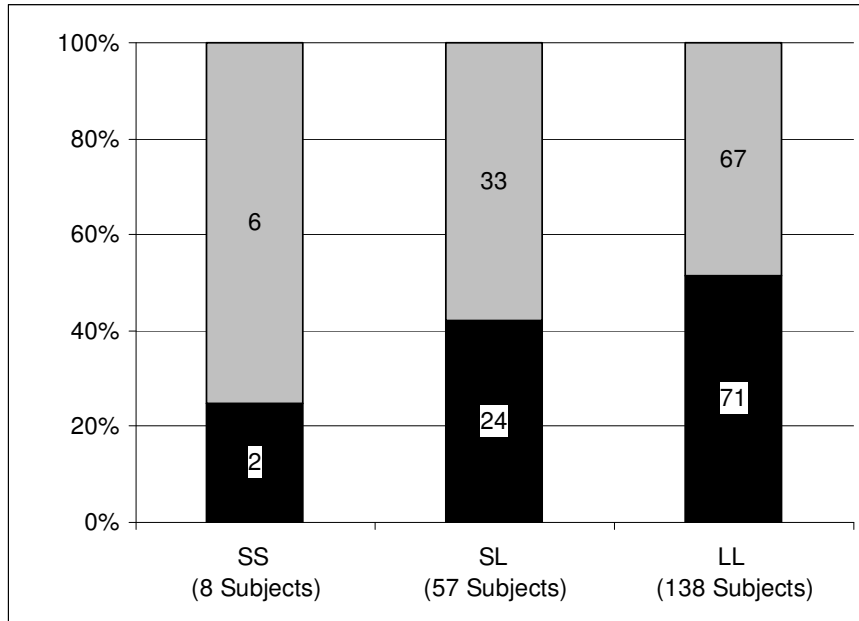
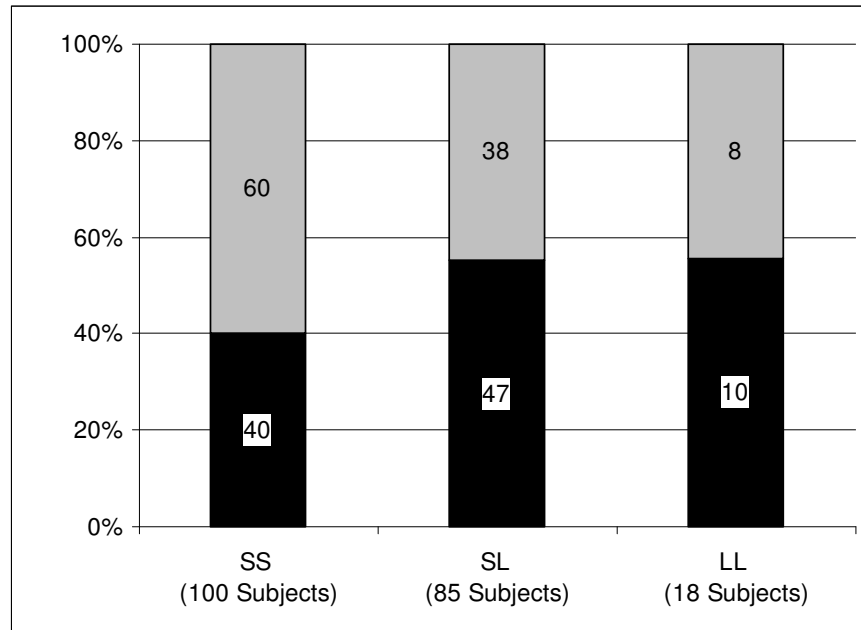
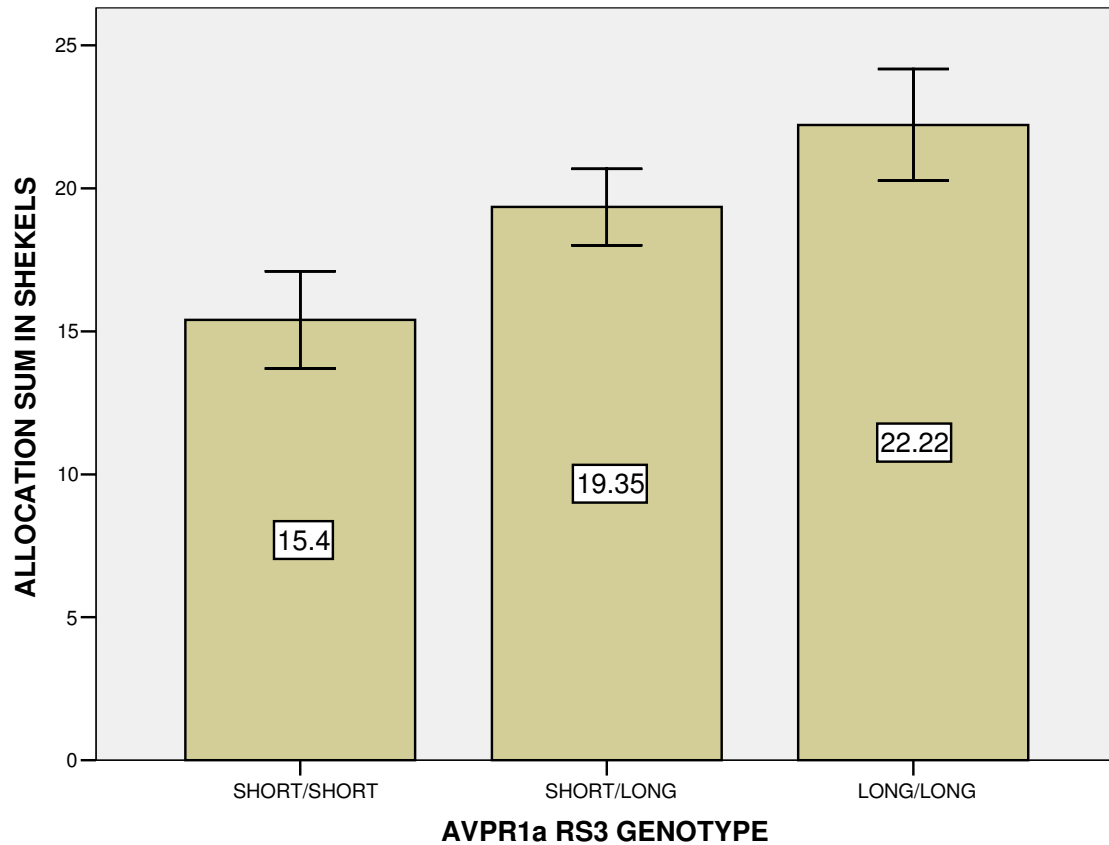


Figure 3c.



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



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

