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ORIGINAL ARTICLE

Genome-wide association study of delay discounting in Heterogeneous Stock rats

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Abstract

Delay discounting refers to the behavioral tendency to devalue rewards as a function of their delay in receipt. Heightened delay discounting has been associated with substance use disorders and multiple co-occurring psychopathologies. Human and animal genetic studies have established that delay discounting is heritable, but only a few associated genes have been identified. We aimed to identify novel genetic loci associated with delay discounting through a genome-wide association study (GWAS) using Heterogeneous Stock (HS) rats, a genetically diverse outbred population derived from eight inbred founder strains. We assessed delay discounting in 650 male and female HS rats using an adjusting amount procedure in which rats chose between smaller immediate sucrose rewards or a larger reward at various delays. Preference switch points were calculated and both exponential and hyperbolic functions were fitted to these indifference points. Area under the curve (AUC) and the discounting parameter k of both functions were used as delay discounting measures. GWAS for AUC, exponential k , and one indifference point identified significant loci on chromosomes 20 and 14. The gene *Slc35f1*, which encodes a member of the solute carrier family, was the sole gene within the chromosome 20 locus. That locus also contained an eQTL for *Slc35f1*, suggesting that heritable differences in the expression might be responsible for the association with behavior. *Adgrl3*, which encodes a latrophilin subfamily G-protein coupled receptor, was the sole gene within the chromosome 14 locus. These findings implicate novel genes in delay discounting and highlight the need for further exploration.

KEYWORDS

adjusting amount, delay discounting, GWAS, Heterogeneous Stock rats

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1 | INTRODUCTION

Delay discounting is the neurobehavioral process by which individuals devalue delayed rewards. It is usually assessed by measuring relative preferences between smaller rewards available immediately and larger rewards with delayed delivery.¹ It has been equated with impulsivity, a multifaceted construct represented by several behavioral phenotypes and linked to substance use disorders (SUD).^{2,3} Recent work has called into question the utility of impulsivity as a unitary construct due to its multifaceted operational definitions.⁴⁻⁷ However, researchers in the field do not dispute the importance of the delay discounting phenotype in SUDs, only whether greater discounting should be interpreted as indicating “impulsiveness.” The lack of controversy regarding the role of delay discounting in SUDs is attributed to the extensive body of evidence accumulated over the last 25 years, with over a hundred published studies comparing delay discounting in drug users and nonusers. Over 80% of these studies have reported higher levels of delay discounting in individuals meeting criteria for SUD, and not a single published study has shown the opposite relationship.^{8,9} Higher levels of discounting have also been associated with other psychopathologies that often co-occur with SUDs, including depression, bipolar disorder, schizophrenia and attention deficit hyperactivity disorder.¹⁰⁻¹² Positive associations have also been reported with pathological gambling,¹³⁻¹⁵ and obesity,^{16,17} suggesting a broader relationship between heightened delay discounting and psychopathology. Indeed, the pervasive association between heightened levels of delay discounting and psychopathology has led some to characterize delay discounting as a “transdisease” or “transdiagnostic” marker and high levels of delay discounting as indicative of a causal “reinforcer pathology.”¹⁸⁻²¹

While the associative link between delay discounting and substance use is well-established, the processes underlying this relationship have not yet been fully elucidated. One contributory mechanism may be common genetic substrates. Familial and twin studies, as well as genome-wide association studies (GWAS), have established that there is a genetic component to delay discounting. Twin studies have shown higher correlations within monozygotic twins compared with dizygotic twins, indicating a strong genetic contribution to the trait.^{22,23} Furthermore, in the largest human GWAS of delay discounting to date, which included 23,127 participants of European ancestry, genotype accounted for 12% of the variance of delay discounting, as measured by the Monetary Choice Questionnaire.²⁴ The heritability of delay discounting in rodents has also been demonstrated using panels of inbred strains.²⁵⁻²⁷

These studies indicate a genetic component to discounting, but only a single gene (*GPM6B*) has ever shown a significant genome-wide association with delay discounting.^{24,28} Other studies have identified risk genes for impulsivity as the broadly defined construct,^{7,28,29} but these genes did not have associations with delay discounting in Sanchez-Roige et al. (2018). This lack of concordance underscores the modest overlap between questionnaire measures of impulsivity and delay discounting, possibly reflecting broader uncertainty over the

true relationship between delay discounting and complex neurobehavioral traits.^{4,5,30} Several studies using animal models have examined the effects of single gene mutants, but with mixed success. No differences were reported between knockouts and wildtypes for *Lphn3*³¹ or *D4* receptor deficiency³²; though reduced delay discounting was reported for conditional knockouts of *Ant1*³³ and augmented discounting was reported following viral vector knockdown of *D2R* localized in the ventral tegmental area.³⁴

Identifying the genes associated with delay discounting may provide valuable information about the transdiagnostic links between discounting and SUDs, or even psychopathologies more generally. Gene identification may suggest novel intervention targets, as well as novel indicators of heightened risk for dysregulated behavior. Furthermore, gene identification may point to critical cell types and neurocircuits that mediate differences in delay discounting and the correlated psychopathologies. Accordingly, the current study aimed to identify genes associated with delay discounting. To accomplish this aim, we phenotyped rats from a Heterogeneous Stock (HS) population. HS rats are an outbred population derived from eight inbred founder strains and have been used extensively for GWAS of other phenotypes.³⁵⁻³⁸ The high level of both genetic and phenotypic diversity of these rats makes this an ideal population to investigate complex neurobehavioral traits such as delay discounting and to identify associated genetic variants.^{39,40}

There is some debate about the most appropriate way to quantify delay discounting.⁴¹⁻⁴³ Changes in relative preference for the smaller, sooner versus the larger, later rewards are typically examined over a series of delays. Traditionally, functions are fitted to the points at which preferences shift at each delay (indifference points) and the slope of this function is used as a measure of delay discounting. Steeper slopes indicate heightened discounting. The function fitted most often is a hyperbolic function from early work by Mazur (1987)⁴⁴ but other functions have also been examined.⁴⁴⁻⁴⁷ Conceptual arguments for hyperbolic functions have included the ability to account for preference switches,⁴⁸ but values that generate preference switches can also be derived for other functions, including the exponential function. Arguments supporting exponential functions have focused on the prevalence of exponential decay in biology. In acknowledgement of the ongoing discussions about the best metrics to quantify delay discounting, and to take advantage of our relatively large dataset, Mitchell et al. (2023) examined the fit of multiple functions based on corrected values for Akaike Information Criterion (AIC).⁴⁹⁻⁵² The hyperbolic and exponential function performed similarly in most cases: AICs were statistically indistinguishable for 63% of the rats ($N = 409$). For the remaining rats, 212 were statistically better described by a hyperbolic function and 29 by an exponential function (33% vs. 4%). This suggests that the exponential function can describe data for individual rats as well as the hyperbolic function for the majority of the rats.⁴⁹ Studies of human delay discounting have also reported that the best fitting function often differs between individuals,^{42,43,53-55} which led to the inclusion of the exponential function in our study. Using this dataset, we adopted three

quantification methods to use in GWAS: the area under the curve (AUC), which is function-free, as well as the slope of the hyperbolic function (hyperbolic k) and the slope of the exponential function (exponential k).^{56–58}

2 | MATERIALS AND METHODS

2.1 | Animals

Subjects were male and female genetically Heterogeneous Stock (HS) rats (official designation: NMcwiWFsm:HS #13673907, RRID: RGD_13673907). HS rats were purchased from Wake Forest University and arrived at Oregon Health & Science University (OHSU) in six shipments between October 2018 and February 2020. Rats from the first four shipments, cohorts 1–4, were phenotyped ($N = 395$). Due to the pandemic lockdown, rats from the fifth and sixth shipments, cohorts 5 and 6, could not be phenotyped but were used as breeders to generate more rats. Breeding took place at OHSU in May 2020 due to the pandemic, with instruction from Dr. Solberg Woods to preserve the genetic diversity and ensure phenotyping occurred with similarly aged rats. The offspring from this breeding were labeled as cohort 7. In total, cohorts 1–4 ($N = 395$) and 7 ($N = 255$) were phenotyped for delay discounting. These cohort groups were used as covariates in the genetic analysis below.

Rats of the same sex were pair-housed with lights on from 6:00 to 18:00 h, at a temperature of 70 °F with ad libitum access to water (except as specified below). They were transported to the laboratory for behavioral testing 5–7 days/week in squads that remained in the laboratory for approximately 2 h. Testing occurred between 9:00 and 17:00 h, that is, during the light phase, and rats were water restricted while in the laboratory. Rats were food restricted starting 1 week prior to the beginning of behavioral training and maintained at approximately 90% ad libitum weight by supplemental feeding immediately after behavioral sessions (PicoLab[®] Laboratory Rodent Diet 5L0D pellets). Weights were monitored daily before behavioral sessions. Rats were treated in compliance with the Guide for the Care and Use of Laboratory Animals⁵⁹ and the experimental protocols were approved by the Institutional Animal Care and Use Committee at OHSU (IACUC; IP00001663).

2.2 | Apparatus

The operant chambers used to examine delay discounting were configured in a similar way to those described previously.⁶⁰ Briefly, the modular rat operant chambers were housed in sound-attenuating chambers (Med Associates Inc., St Albans VT, USA). On one wall of the chamber there were two nonretractable levers, with a stimulus light above and a liquid receptacle below each. Between the levers was a nose poke with a light. On the opposite wall there was a speaker-tone generator combination and a clicker. Two 3.33 rpm syringe pumps were used to deliver 10% w/v sucrose solution to each

of the liquid receptacles inside the chamber. MED-PC V software (1 ms resolution) was used to control the equipment and record activity. Operation of the equipment was tested prior to sessions.

2.3 | Delay discounting assessment

After training, rats were exposed to the adjusting amount procedure (Figure 1A), as adapted from Richards et al. (1997), and used extensively with rats.^{26,61–65} Briefly, sessions included free- and forced-choice trials, and ended after 60 free-choice trials occurred, or 60 min had elapsed. On free-choice trials, the size of the reinforcer delivered when the delay lever was pressed was 150 μ L (A_D , Figure 1A), while the reinforcer associated with the immediate lever was adjusted throughout the experimental session (A_I , initial size: 75 μ L, Figure 1A). Choice of the delay lever increased the current size of the immediate reinforcer by 10% on the following trial to a maximum 300 μ L. Choice of the immediate lever decreased the current size of the immediate reinforcer by 10% on the following trial to a minimum of 5 μ L. The size of the immediate reinforcer was not altered following forced-choice trials, which occurred after two consecutive choices of either the delay or immediate lever. Variable length timeouts between trials ensured that trials occurred every 30 s, regardless of the choice on the previous trial. Choice of the delay lever resulted in reinforcer delivery after 0, 2, 4, 8, 16, or 24 s. This delay remained constant within a session but varied between sessions according to a Latin Square design that was the same for all rats. Rats experienced each delay on 6 occasions.

2.4 | Statistical analysis for phenotyping

For each rat, an “indifference point” was calculated for each delay (T_s), which represents the point where the subjective value of the delayed but fixed reward (A_D) is equal to the adjusting amount (A_I). The median A_I value from the last 30 trials of each session was obtained and the values averaged over the six sessions for each delay.⁴⁹ This resulted in one indifference point value for each delay time, which is plotted for each animal (Figure 1B). Intraclass correlation (ICC) was used to assess the reliability between the six sessions for each rat. ICC was computed using a two-way mixed-effects model. The ICC coefficient was 0.6 (95% CI 0.524 < ICC < 0.648, $p = 2.36e-37$), indicating a moderate level of agreement across sessions, which is consistent with human data.⁶⁶

We used two procedures to enhance the robustness of our indifference point measures. First, we excluded any session on which fewer than 45 of the 60 free-choice trials were completed by a rat: 1714 out of 23,400 (7.3%) sessions (650 rats \times 6 delays \times 6 occasions). Second, we excluded any completed session on which choices during the second half of the session were primarily on one lever (operationalized as 80% or more of trials 31–60 during the session): 967 out of the 21,686 (4.5%) completed sessions. These exclusions resulted in 20,719 sessions of data from which indifference points

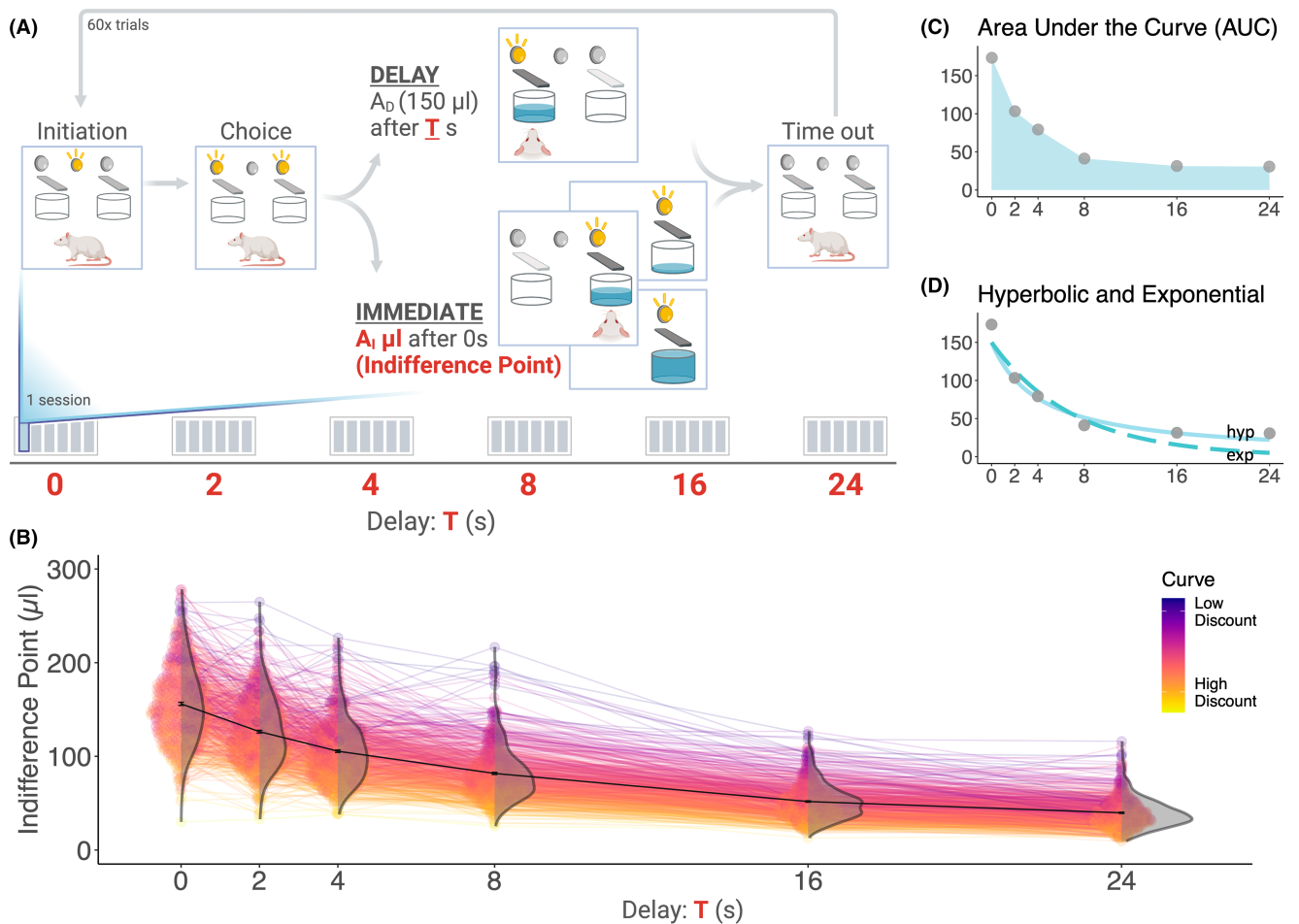


FIGURE 1 Multiple indices of delay discounting were calculated for each HS rat ($n = 629$). (A) Schematic of the adjusting amount procedure, where one session for a specific delay includes ~ 60 trials, and rats are exposed to six sessions per delay. Indifference points for each delay (T s) were calculated based on the A_i values from the last 30 trials of each session. (B) Indifference points were plotted for each delay to create discounting curves for each individual rat (one line per rat). Low to high discounting, based on the steepness of the discounting curve for a rat, is denoted by color, with a low discounting rat having a darker purple curve and a high discounting rat having a brighter yellow curve. The black curve connects the mean values for each delay showing the average delay discounting curve for all rats. Error bars represent standard error. Violin plots show the distribution of indifference points for rats at each delay. (C–G) Examples for delay indices are represented with a curve for a single rat. (C) Area under the curve (AUC) was calculated for each rat by summing the area of the trapezoids created by the indifference points. (D) Hyperbolic and exponential functions were fitted to the curve for each rat, and the k parameter from each function was used as a trait in GWAS.

were derived. These indifference points provide an index of the subjective value of the 150 μl 10% sucrose solution and are expected to decrease as the delay to receiving the 150 μl reward increases, reflecting the putative decrease in its subjective value.

Two strategies were used to quantify the extent of decreases in subjective value. First, we fit either a hyperbolic⁴⁴ or an exponential⁶⁷ mathematical function to the indifference points for individual rats:

$$\text{Hyperbolic: } V = \frac{bA_D}{1+kT} \quad (1)$$

$$\text{Exponential: } V = bA_D e^{-kT} \quad (2)$$

V represents the subjective value at the indifference points, b represents an individual's side preference in the apparatus, the A_D

represents the amount of the larger reward (150 μl), T represents the time delay to that reward, k represents the discounting parameter (slope of the function). For both the hyperbolic and exponential functions, bias was assumed to be equal to 1, where there were no differences in side preference for each animal. Side bias is not included in any assessments of delay discounting used with human participants; however, Mitchell et al. (2023) calculated this bias by dividing the indifference point at delay 0 s by 150 μl , to generate a unit free constant that adjusts the curve starting point at delay 0.⁴⁹ Bias accounted for any preference for the left or right lever, and preferences for each were roughly equal across rats. The inclusion of bias affects the fit of both functions, and all parameters for functions with and without bias for all rats used here can be found on Github (https://github.com/Palmer-Lab-UCSD/HSrat_delaydiscounting). The models that account

for bias are better fit than assuming no bias, but the numerical value of the slopes of both functions are altered and the genetic basis for side bias remains unclear. The underlying drivers of bias may be multifactorial⁶⁴ and including a side bias constant does alter the derived hyperbolic and exponential slopes. Because the underlying genetic basis of bias is still opaque, we used the functions without bias for the remainder of this study, and we include the results of the bias models from GWAS in Figure S1. Additionally, there were no significant loci associated with exponential or hyperbolic indices with bias.

We also conducted assessments of model fit to ensure the appropriateness of computing model parameters (k) for all rats, which is described in depth in Mitchell et al. (2023). Briefly, to evaluate the fit of both the hyperbolic and exponential functions, we employed sum of squared residuals and the coefficient of determination, which provide quantitative assessments for how well each model captured the observed discounting behavior at each indifference point. While model fit varied across rats, we ensured the quality of the data for each rat by filtering for clean experimental data and processing covariate effects as described below. The k parameter was ultimately used for all animals as it allowed us to capture the range of discounting behaviors present. Even in modest cases of poor model fit, the estimation of model parameters can still provide information into the underlying discounting processes and allow for meaningful comparisons. Furthermore, we used two models as well as area under the curve (AUC) to ensure robustness of the discounting phenotype for GWAS.

Second, we calculated the AUC by summing the areas of the trapezoids created by indifference points.⁵⁷ Taken together, AUC, exponential and hyperbolic k , as well as the indifference points at each delay were all used to index the delay discounting trait for GWAS (9 measures total). Analysis for delay discounting was done in *R* and can be reproduced using the pipeline available on Github (https://github.com/Palmer-Lab-UCSD/HSrat_delaydiscounting).

2.5 | Genotyping

A total of 650 experimental HS rats were genotyped. Spleens were collected postmortem and used as a source of DNA for genotyping ([dx.doi.org/10.17504/protocols.io.6qpvr665ovmk/v1](https://doi.org/10.17504/protocols.io.6qpvr665ovmk/v1)). Spleen tissue samples were cut and processed ([dx.doi.org/10.17504/protocols.io.36wqg7nryvk5/v1](https://doi.org/10.17504/protocols.io.36wqg7nryvk5/v1)), and DNA was isolated using the Beckman Coulter DNeasy Advance Kit at the University of California San Diego ([dx.doi.org/10.17504/protocols.io.8epv59reng1b/v1](https://doi.org/10.17504/protocols.io.8epv59reng1b/v1)). All samples were normalized and processed in a randomized order prior to library preparation ([dx.doi.org/10.17504/protocols.io.261genw5dg47/v1](https://doi.org/10.17504/protocols.io.261genw5dg47/v1)), and multiplexed sequencing libraries were prepared using the iGenomX RipTide kit ([dx.doi.org/10.17504/protocols.io.j8nlkkm8515r/v1](https://doi.org/10.17504/protocols.io.j8nlkkm8515r/v1)). Final QC was performed on sequencing libraries; sequencing was performed using an Illumina NovaSeq 6000 ([dx.doi.org/10.17504/protocols.io.yxmvnmw29g3p/v1](https://doi.org/10.17504/protocols.io.yxmvnmw29g3p/v1)). Reads were demultiplexed using *fgbio* v1.3.0 (<http://fulcrumgenomics.github.io/fgbio/>) before trimming adapters using *BBDuk* v38.94 (<https://sourceforge.net/projects/bbmap/>) and quality trimming using *Cutadapt* v4.1.⁶⁸ Reads were aligned to the rat

reference genome mRatBN7.2 from the Rat Genome Sequencing Consortium (GCA_015227675.2 GCF_015227675.2) using *BWA-mem* v0.7.17.⁶⁹

Mapped sequences were then used to construct haplotypes and impute biallelic SNP genotypes using *STITCH* v1.6.6.⁷⁰ From this set of 10,684,883 SNPs, we removed all SNPs with low imputation quality scores produced by *STITCH* (INFO < 0.9; 2,609,890 SNPs removed). We additionally removed all SNPs with high missing rates (missing rate > 0.1; 21,900 removed), low minor allele frequencies (MAF < 0.005; 2,600,296 removed), and extreme deviations from Hardy-Weinberg Equilibrium (HWE $p < 1e-10$; 2370 removed). This filtered set of 5,451,257 SNP genotypes was used for all downstream analyses.

2.6 | Phenotype data processing and genetic analysis

While the AUC data were relatively normally distributed (skew = 0.80, kurtosis = 1.30), the exponential and hyperbolic values were not (exponential k skew (SE): 3.20 (0.10), kurtosis (SE): 20.57 (0.20); hyperbolic k skew: 3.12 (0.10); kurtosis: 20.44 (0.20)). In human studies, log transformations are commonly applied to these values. However, in order to address these deviations from normality, as well as interpret results from GWAS across traits for rats and have comparable significance thresholds, all delay discounting traits (including the three discounting indices and the six individual indifference points) were quantile normalized. To address any differences between sex, values for traits for males and females were first split, then quantile normalized. This creates identical distributions for both males and females and equalizes any effects. In addition, we consider the following as covariates: cohort, coat color, cage, and age. To examine the effects of these covariates, we fit linear models that predicted the phenotype based on each distinct covariate. We used linear regression to remove the effects of covariates that explained more than 2% of the variance of the trait. This is intended to capture and remove extraneous information such as local breeding versus shipping, pandemic effects, different technicians, or seasonal differences. For AUC, and exponential and hyperbolic k , the four separate shipments of rats (cohort 1–4) as well as the final group of rats bred at OHSU (cohort 7), explained more than 2% of the variance of these indices, so they were regressed out. Once all traits are quantile normalized, and males and female values are recombined, their distributions are identical and normal, and no other transformations are required to run GWAS or permutation tests (below).

Phenotypic correlations between the three derived measures of discounting (AUC, exponential k , and hyperbolic k) and the six indifference points for each delay (Delay 0, 2, 4, 8, 16, and 24) were determined using Pearson's correlation to test the strength of the linear relationship between measures. These correlations were visualized using Seaborn Clustermap. Average linkage hierarchical clustering for the traits was also utilized to explore patterns of similarity among the three derived measures of discounting and six indifference points

(9 measures total). Average linkage hierarchical clustering considers the distance between clusters based on the average pairwise distance between members, resulting in clusters with similar average distances to each other.

GWAS analysis was performed using the mixed linear model analysis (MLMA) function from the Genome-wide Complex Trait Analysis (GCTA) software package to compute association statistics explaining the genetic contribution to phenotypic variance.⁷¹ This algorithm builds a genetic relationship matrix (GRM) between individuals and uses the leave one chromosome out (LOCO) method, which leaves out SNPs on the same chromosome as the test SNP to avoid proximal contamination.⁷² SNP heritability estimates were obtained using the restricted maximum likelihood (REML) approach from the GCTA package, which relies on the GRM to estimate the proportion of phenotypic variance explained by all SNPs.⁷¹ Genetic correlations between traits were estimated using bivariate GCTA-REML analysis. These correlations were also visualized and clustered using hierarchical clustering.

Genome-wide significance thresholds ($\alpha = 0.05$ and 0.10) were calculated using permutation tests.^{36,73} The same thresholds were used for all delay discounting indices because all phenotypes were quantile normalized and thus had identical genotypes and identical phenotypic distributions. We report all SNPs with p -values exceeding the significance threshold of $-\log_{10}(p) = 5.58$ ($\alpha = 0.05$) or $-\log_{10}(p) = 5.36$ ($\alpha = 0.10$).

GWAS was only performed for rats for which all delay discounting indices could be obtained ($n = 629$, females = 319, males = 310), including AUC, and exponential and hyperbolic k . Additional analyses were also conducted for the indifference points at each delay (0, 2, 4, 8, 16, 24 s).

Each chromosome was scanned to identify quantitative trait loci (QTLs) containing at least one SNP that exceeded the threshold. Linkage disequilibrium (LD) intervals for each significant QTL were determined by finding additional significant SNPs within 0.5 Mb that had a high correlation ($r^2 = 0.6$) with the peak SNP. We generated a porcupine plot combining the Manhattan plots for the traits showing QTLs of genome-wide significance, as well as Regional Association Plots for the significant QTLs for each trait.

3 | RESULTS

3.1 | HS rat phenotyping for delay discounting

Adult HS rats completed the adjusting amount procedure to measure delay discounting (Figure 1A) and indifference points were recorded for each time delay. Due to missing data from incomplete sessions, indices of delay discounting could not be calculated for 21 genotyped rats, resulting in a total of 629 rats (females = 319, males = 310) with complete delay discounting profiles. Indifference points were plotted to generate discounting curves for each rat (Figure 1B). Data from 87% of rats was systematic (criterion 1: indifference point was >20%

larger than the preceding point),⁷⁴ but data from all rats were included in the analyses because, for each rat, systematicity violations were only single indifference points. Discounting curves for rats ranging from low to high discounting are denoted by color. For each rat discounting curve, several indices were calculated as measures of delay discounting, including area under the curve (AUC, Figure 1C). Hyperbolic and exponential functions were also fit to indifference points (Figure 1D). The discounting parameter, k , was calculated for the exponential and hyperbolic functions for each rat.

These three values (AUC, exponential k , and hyperbolic k) were used as the measures of delay discounting in GWAS. We also included the indifference points at each delay in the genetic analysis to determine if the individual components making up the delay curve were driving the results. Nine total measures were used as traits for GWAS. Strong phenotypic correlations were found between the exponential and hyperbolic k parameters, while AUC had stronger phenotypic correlations with the indifference points and clustered separately (Figure 2A). Though there were nine phenotypic indices total, two phenotypic clusters emerged due to strong correlations between the measures. Genetic correlations followed a similar pattern of clustering (Figure 2B). SNP heritability estimates ranged from 0.08 ± 0.05 to 0.19 ± 0.06 (Figure 2B). The three delay discounting indices (AUC, exponential k , and hyperbolic k) had higher heritability estimates than the individual indifference points at each delay (Figure 2B, Table S1). Heritability estimates for AUC, exponential k , and hyperbolic k were consistent with many other behavioral traits that have been reported previously in HS rats in the past. However, estimates for the indifference points at each delay were generally lower than the composite delay discounting indices.

3.2 | GWAS for delay discounting

After filtering and controlling for quality as described in the Methods section, we obtained genotypes at 5,451,257 SNPs for 629 rats. We performed a GWAS of delay discounting indices for AUC, exponential k , and hyperbolic k parameters for 629 rats. Indifference points for each delay were also subsequently included in the GWAS.

We detected a genome-wide significant locus on chromosome 20 for both the AUC and exponential k delay curve indices (Figure 3A–C). GWAS identified the same top SNP (20:32,221,020) in the locus for AUC and exponential k , which had a minor allele frequency of $\sim 17\%$. The minor allele was derived from BN/N and ACI/N, whereas the other 6 founders had the major allele. The minor allele was associated with higher discounting, as demonstrated by lower AUC (Figure 3E) and higher values of exponential k (Figure 3F). For AUC, the top SNP showed a $-\log_{10}(p) = 5.689$, which corresponds to a $p < 0.05$. For exponential k , the same SNP showed a $-\log_{10}(p) = 5.449$, which corresponds to $p < 0.10$. For hyperbolic k , this SNP showed a $-\log_{10}(p) = 4.915$, which was near threshold but not significant. A porcupine plot combining the two traits is shown in Figure 3A. This top SNP is located in an intron of the gene *Sc135f1* as depicted in

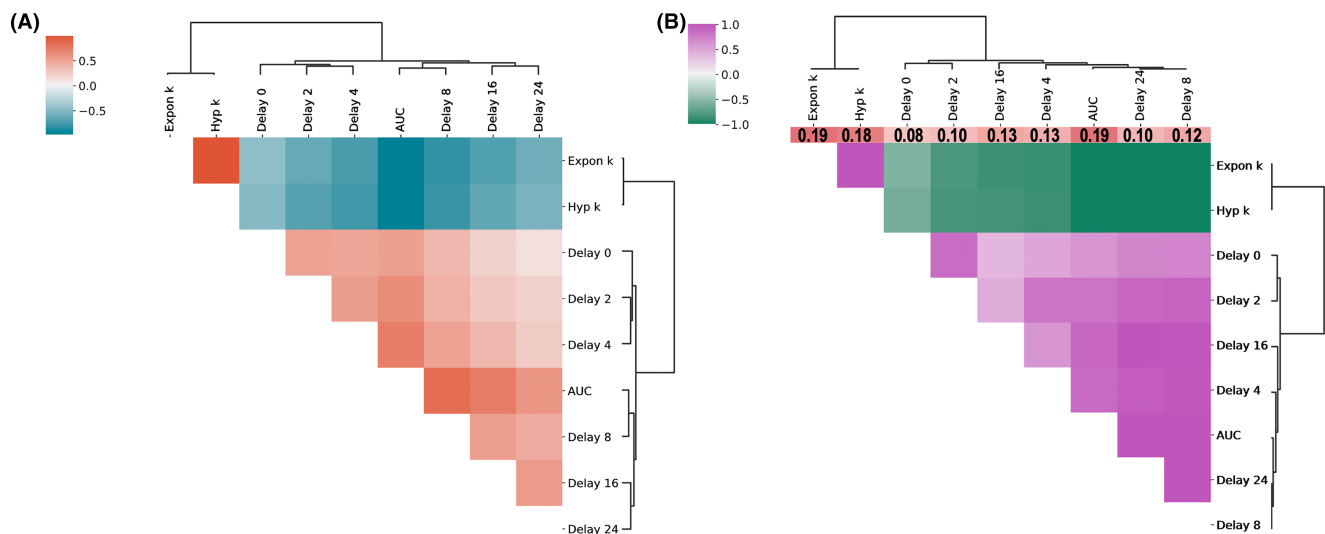


FIGURE 2 Phenotypic and genetic correlations were calculated for all delay discounting measures. (A) Phenotypic correlations were determined using Spearman's test, and traits were clustered using average linkage hierarchical clustering. (B) Genetic correlations were calculated using bivariate REML analysis implemented in GCTA. SNP heritability estimates for each trait are denoted in the color bar above the heatmap.

the locus zoom plots in Figure 3B,C, and the nearby SNPs in this QTL that are in strong linkage disequilibrium (LD) with the top SNP ($r^2 > 0.8$) are located in multiple exons and introns of *Slc35f1*. *Slc35f1* encodes a member of the solute carrier family 35, which has been implicated in brain-related function and neurodevelopmental disorders. There are two expression QTLs (eQTL) for *Slc35f1* (20:32,306,446 and 20:32,306,658) that reflect heritable differences in expression of *Slc35f1* in whole brain and prelimbic cortex. These eQTLs are in strong LD with the top SNP ($r^2 = 0.97$), suggesting that heritable differences in the expression of *Slc35f1* may mediate the effect of this locus on delay discounting. We did not identify any other eQTLs in this locus nor were there any coding variants that were predicted to have major effects of in this locus.

GWAS also detected a genome-wide significant locus on chromosome 14 for the indifference point at the 2 s delay (Figure 3A). The SNP 14:26,702,994 ($-\log_{10}(p) = 5.496$, $p < 0.10$) had a minor allele frequency of $\sim 21\%$ and the major allele was derived from MR/N, with all other founders having the minor allele. Lower indifference points for the short 2 s delay were associated with the minor allele (Figure 3G). Of note, this SNP neared genome-wide significance for the indifference point at 0 s delay ($-\log_{10}(p) = 4.418$). This SNP is located in an intron of the gene *Adgrl3*, which encodes a type of G-protein coupled receptor. Additionally, multiple eQTLs exist in the chromosome 14 locus for *Adgrl3* for various tissues including brain (14:26,678,469), infralimbic cortex (14:26,745,338), and lateral habenula (14:26,724,419), which all were in high LD with the top SNP ($r^2 > 0.96$). Multiple other eQTLs for *Adgrl3* exist in this locus that are also in LD with the top SNP, but to a lesser degree ($r^2 \sim 0.6$), and these include: basolateral amygdala (14:26,776,241), nucleus accumbens (14:26,782,299, 14:26,779,935), orbitofrontal cortex (14:26,776,241), and prelimbic cortex (14:26,790,378, 14:26,776,241).⁷⁵

4 | DISCUSSION

The main aim of the present study was to identify genes associated with delay discounting using genetically heterogeneous rats. To accommodate the lack of consensus about discounting curve models, delay discounting was indexed in multiple ways including fitting a hyperbolic and an exponential function to the indifference points. The hyperbolic equation, and variants of this equation family, has been suggested as a conceptually more attractive way to describe delay discounting than the exponential function, though that is a discussion beyond the scope of this manuscript.^{76–78} Because the appropriate summary measures for delay discounting is not settled science, the area under the indifference points (AUC) was calculated. GWAS for these indices of delay discounting, as well as indifference points at each delay, identified two genome-wide significant QTLs located on chromosome 14 and 20, despite the relatively small sample size. The gene *Slc35f1*, which encodes a member of the solute carrier family of membrane transport proteins,⁷⁹ was the only gene within the chromosome 20 locus; and the *Adgrl3* gene, which encodes a member of the latrophilin family of G-protein coupled receptors,⁸⁰ was the only gene within the chromosome 14 locus. There were also multiple eQTLs for tissues in the brain for *Slc35f1* and *Adgrl3* that were in high LD with the top SNPs for these loci ($r^2 > 0.96$),⁷⁵ meaning that these loci also change expression of *Slc35f1* and *Adgrl3*, which may be driving the observed behavioral differences.

SNP heritabilities, or the proportion of variance accounted for by SNPs, for the three delay discounting indices AUC, exponential k , and hyperbolic k were estimated to be 19% (SE = 0.06), 19% (SE = 0.06), and 18% (SE = 0.06), respectively. Heritabilities for the indifference points at each delay were lower comparatively, ranging between 8% to 13%. This difference may arise because the delay discounting behavior is better described by the composite measures, which

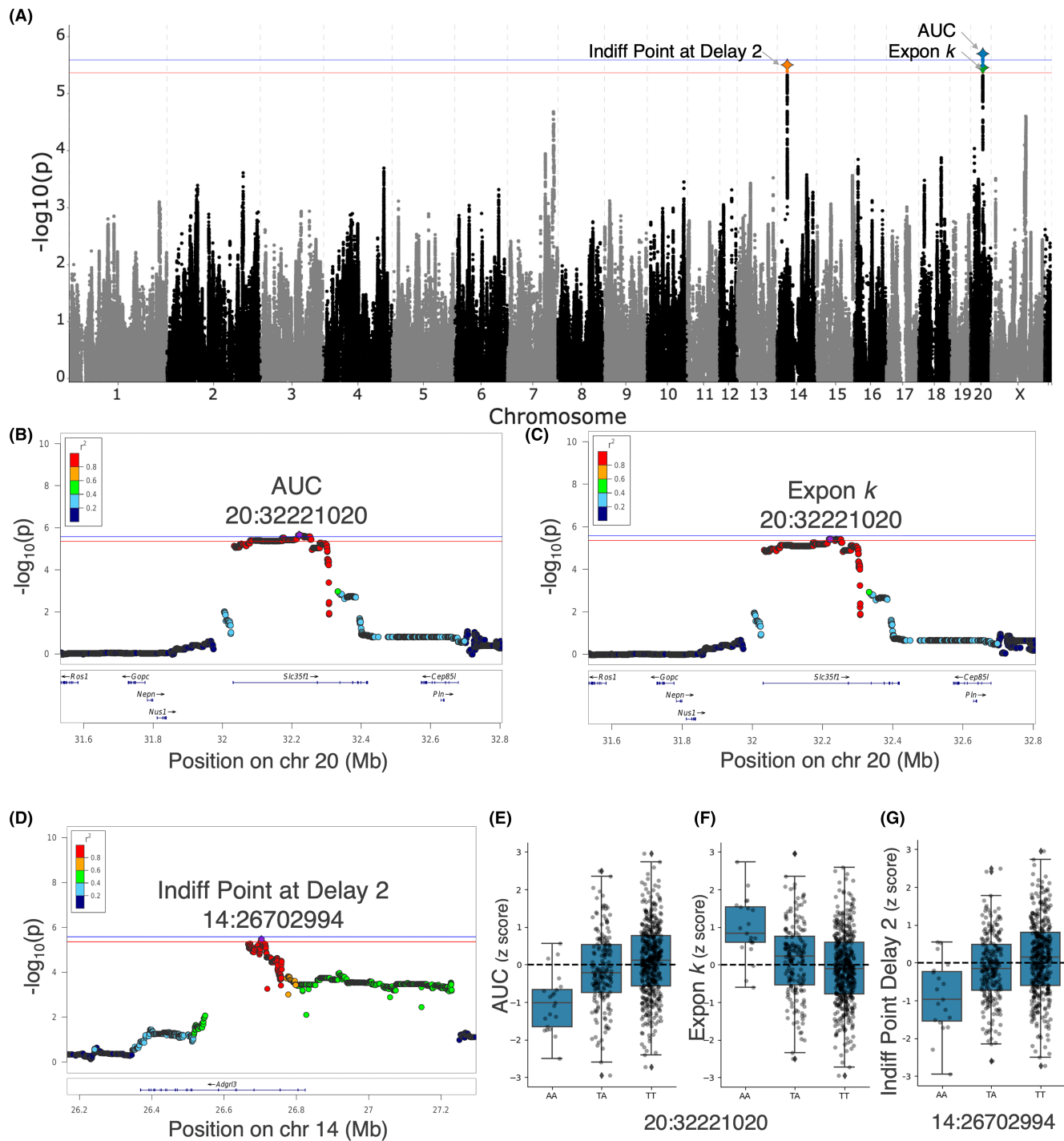


FIGURE 3 GWAS for AUC and exponential k measures for delay discounting, as well as the indifference point at the 2 s delay resulted in significantly associated loci mapping to the two genes: *Slc35f1* and *Adgrl3*. (A) The porcupine plot displaying the chromosomal distribution of all p -values combines the three delay discounting measures and shows both significant chr 20 SNPs and the chr 14 SNP. The blue and red lines show the significance thresholds derived from the permutation tests: $-\log_{10}(p) = 5.58$ ($\alpha = 0.05$) and $-\log_{10}(p) = 5.36$ ($\alpha = 0.10$), respectively. (B–D) Regional association plot for the QTL on chr 20 for both delay discounting indices: AUC and exponential k , and for the QTL on chr 14 for indifferent point at 2 s delay. The x-axis depicts chromosomal position in Mb and the y-axis shows the significance of the association ($-\log_{10}(p)$), and individual dots represent SNPs. Purple denotes the top SNP associated with the trait, and color of the dots indicate level of linkage disequilibrium between top SNP and other nearby SNPs. (E–G) Effect plots for all three measures showing the genetic effect of the peak SNP. The minor allele for the chr 20 SNP was associated with heightened discounting with lower AUC and higher exponential k values, and the minor allele for the chr 14 SNP was associated with lower indifference points at 2 s delay.

capture the aggregate effects of the genetic factors underlying delay discounting compared with the individual indifference points. Regarding the models that incorporated a bias term (accounting for any preference for the left or right lever), including exponential and hyperbolic k with bias, heritabilities were also lower, ranging between 8% and 12% (Table S1). There were also no significant loci associated with exponential or hyperbolic indices with bias (Figure S1). The difference in heritability among the discounting indices with and without bias is of note, as the inclusion of the bias term in both functions increases variance accounted for, but simultaneously reduces heritability estimates. The underlying genetics of side bias remain opaque and appear to introduce noise making the genetic bases of delay discounting more difficult to distinguish. Here, we report the significant findings from GWAS for delay discounting inclusive of the exponential and hyperbolic models because they are further supported by the findings using the function-free index: AUC.

As expected for SNP heritabilities, these heritabilities were lower than estimates derived from inbred strains, as anticipated. Higher genetic homogeneity in strain-based studies allows for greater estimation of genetic effects and thus higher heritabilities compared with this study in an outbred rat population that has much higher genetic variation. For example, Wilhelm and Mitchell (2009) reported 40% heritability for hyperbolic k with bias in six inbred strains of male rats using the adjusting task described here (measures without bias were not reported), and Richards et al. (2013) reported 50% heritability for AUC in eight inbred strains of male rats using a nonstandard delay discounting task. Lower estimates have been reported in mouse studies. Isles et al. (2004) estimated heritability to be 16% for a small immediate versus larger later choice preference measure based on four inbred strains of male mice.⁸¹ In another more recent screen of heritable variation in delay discounting, Bailey et al. (2021) used male and female mice from the highly genetically diverse Collaborative Cross (CC) recombinant inbred panel of mice as well as the eight founder strains from which all CC mice are derived.²⁵ The combined 18 strains demonstrated significant heritability for a proxy measure of delay discounting with 25% of the variance explained by strain differences.

4.1 | *Slc35f1*

GWAS for AUC and exponential k identified the same significant top SNP that mapped to the gene *Slc35f1*, which encodes a member of the solute carrier (SLC) family of membrane transport proteins.⁷⁹ The SLC35 family of nucleoside-sugar transporters were thought to localize in the Golgi apparatus and endoplasmic reticulum (ER)⁸²; however, a more recent study of SLC35F1 protein expression in the adult mouse forebrain did not find co-localization of *Slc35f1* with the Golgi apparatus or ER.⁸³ Nevertheless, *Slc35f1* did have high expression in the soma and dendrites of neurons in numerous cortical and diencephalic structures including the hippocampus and thalamus.⁸³ The authors suggested the possible involvement of *Slc35f1* in the formation and function of dendritic spines or synaptic plasticity. High SLC35F1 mRNA expression has also been found in both fetal and

adult human brain tissues.⁸⁴ Consistent with the protein expression patterns in the murine study, the SLC35F1 protein as well as RNA is highly expressed in multiple brain regions in human tissue including the cerebral cortex, hippocampus, and amygdala ([proteinatlas.org](https://www.proteinatlas.org)).^{85,86} The high neuronal expression and potential role in dendritic spine dynamics point to a brain-related function, yet the full molecular mechanisms by which SLC35F1 participates in neuropsychiatric behaviors and substance use disorders (SUDs) remains unresolved.

In humans, there is some evidence to suggest that SLC35F1 is involved in critical brain pathways underlying behavior and/or neuropathophysiology, which may give some clues about its association to delay discounting. First, Szafranski et al. (2015) described six unrelated pediatric epilepsy patients with microdeletions within a ~5 Mb region on 6q22.1q22.23.⁸⁷ They narrowed the critical region to a segment that included a putative cis-regulatory sequence of the SLC35F1 promoter and a portion of the SLC35F1 gene itself, among other regulatory and gene sequences. Importantly, patients with microdeletions spanning this SLC35F1 regulatory region had a constellation of varying presentations including multiple types of recurrent or refractory epilepsy, autism spectrum disorder, speech and language delay, abnormal EEG, cognitive delay, developmental regression, intellectual disability, tremor, and global delay. Additionally, Fede et al. (2021) recently described a patient with an SLC35F1 gene mutation who exhibited a Rett syndrome (RTT)-like phenotype where she experienced refractory seizures, had severe intellectual disability and limited speech, and was unable to walk independently.⁸⁸ Together, these genetic case studies suggest an important neurodevelopmental role for SLC35F1.

Several human GWAS for neurological and psychiatric phenotypes have also implicated SLC35F1. In a GWAS meta-analysis of attention-deficit/hyperactivity disorder (ADHD) and bipolar disorder, SNPs annotated to SLC35F1 neared significance, but were subthreshold (best $p = 6 \times 10^{-8}$).⁸⁹ Interestingly, however, the strongest SNP identified in this study was rs11756438, which was in LD with SNPs in the SLC35F1 gene. Another SNP located in SLC35F1 also neared significance ($p = 3 \times 10^{-6}$) in a recent GWAS of schizophrenia,⁹⁰ which is noteworthy in light of the known connection between steeper discounting and schizophrenia.¹⁰ Furthermore, in an updated GWAS meta-analysis of educational attainment with about three million individuals, SNPs rs11755280 and rs12213071 located in the SLC35F1 gene were significantly associated with educational attainment ($p = 2 \times 10^{-9}$ and $p = 1.26 \times 10^{-9}$, respectively).⁹¹ Four other SNPs mapped to genes in the SLC35 family were also significant, including SLC35D2, SLC35F4, and SLC35F5. Importantly, lifetime outcomes such as educational attainment have been negatively associated with delay discounting, where individuals with steeper delay discounting have lower educational attainment.⁹²

Genetically altering SLC35F1 in murine model systems has been less successful at recapitulating the phenotypes observed in humans. Deletion of *Slc35f1* in mice did not result in any phenotypic outcomes related to the RTT-like syndrome described by Fede et al. (2021), nor the microdeletion syndrome described by Szafranski et al. (2015).⁹³ However, those studies did not assess delay discounting or similar behavioral constructs.

4.2 | *Adgrl3*

GWAS for the indifference point at the 2 s short delay identified a genome-wide significant locus on chromosome 14 that mapped to the gene *Adgrl3*, and there were several eQTLs for *Adgrl3* at loci in strong LD with the top SNP. *Adgrl3*, also called *Lphn3*, encodes a member of the latrophilin (LPHN) subfamily of G-protein coupled receptors.⁸⁰ The human homolog has been reliably associated with ADHD,^{94,95} and has been suggested to confer susceptibility to SUDs using a tree-based predictive analysis.⁹⁶ Importantly, delay discounting, ADHD, and SUDs are all genetically correlated with one another and often co-occur.^{24,97,98} Functionally, *ADGRL3* is involved in synapse development in the cortex⁹⁹ and is highly expressed in the brain (proteatlas.org).⁸⁶ Unfortunately, as mentioned before, in a study using a delay discounting task where rats were given an option between immediate food pellets or delayed, but a larger number of food pellets, *Lphn3* knockout rats did not show any differences in what the author's term "impulsive choice" compared with wildtype controls.³¹ However, in a different rat study, *Lphn3* knockout in Sprague–Dawley rats did result in hyperactivity, increased acoustic startle response, and reduced activity in response to amphetamine administration.¹⁰⁰ Furthermore, loss of the homologous gene (*lphn3.1*) in zebrafish resulted in ADHD-like behavior and abnormal development of dopaminergic neurons,¹⁰¹ and *Lphn3* null mutant mice showed hyperactivity and increased sensitivity to cocaine.¹⁰² More recently, however, *Adgrl3* knockout in mice on a B6/J background showed no neuro- or behavioral differences,¹⁰³ though delay discounting was not assessed.

Adgrl3 has also been associated to multiple traits in human GWAS. Several variants have been associated with educational attainment, including in a recent GWAS using data from the UK Biobank ($p = 5 \times 10^{-11}$).^{104,105} *Adgrl3* has also been associated with risk-taking behavior ($p = 1 \times 10^{-9}$),¹⁰⁶ smoking initiation ($p = 5 \times 10^{-9}$),¹⁰⁷ and externalizing behavior ($p = 5 \times 10^{-9}$).¹⁰⁸ Considering its association to ADHD and potential SUD risk, as well as evidence in model organisms for its involvement in neuropsychiatric-related behaviors, *Adgrl3* is a strong candidate for further study.

4.3 | Limitations, conclusions, and future directions

While our study yielded intriguing results that will form a foundation for future research on the genetics of delay discounting, the work is limited by its small sample size. This may have contributed to our inability to replicate the findings of the recent human GWAS of delay discounting identified a significant association with the SNP rs6528024, which is located on the X chromosome in an intron of the neuronal membrane glycoprotein M6B gene (*GPM6B*).²⁴ In a follow-up study, *Gpm6b* deletion in C57BL/6J mice resulted in an increased preference for smaller immediate rewards compared with larger delayed rewards, reflecting higher delay discounting.¹⁰⁹ A small, preliminary rat study, using CRISPR to delete *Gpm6b*, was consistent with these data ($p = 0.18$) (Mitchell, personal communication).

However, it is also possible that HS rats do not have sufficient variability in *Gpm6b* expression, in which case we would not detect *Gpm6b* even if it is truly associated with delay discounting in other populations or species. Conversely, no human GWAS of delay discounting has detected a significant association with *SLC35F1* or *ADGRL3*. This may be attributable to our identification of these loci being based on different indices of delay discounting (AUC and exponential k),¹¹⁰ or to a lack of sufficient variation in *SLC35F1* or *ADGRL3* among humans. There is limited literature, but SNPs that have been identified in selected lines that differ in both delay discounting and substance use were not identified (e.g., High Alcohol Drinking and Low Alcohol Drinking rats).^{60,65,111} Furthermore, we acknowledge that these loci do not comprehensively explain all differences in delay discounting, and the behavior is polygenic. The functional interpretation of these genes coincides with the neurobehavioral implications of their identification through GWAS, and the preliminary nature of these results warrants further investigation. Other differences between assessments of delay discounting in human and rodent studies may also be a factor in the lack of concordance, for example, the use of hypothetical versus real rewards in human versus rodent studies, the time scale over which discounting is assessed, as discussed by numerous authors.^{112,113} These methodological differences are difficult to address but explorations of whether such moderating effects are genetically influenced could be the focus of future studies. Finally, while not identified at genome wide significant levels, we assume that additional loci can and will be identified in the future, once larger sample sizes are available.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in HSrat_delaydiscounting at https://github.com/Palmer-Lab-UCSD/HSrat_delaydiscounting.

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