Genome-Wide Association Study in a Rat Model of Temperament Identifies Multiple Loci for Exploratory Locomotion and Anxiety-Like Traits.

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INTRODUCTION

We probed genetic differences produced by a long-term selective breeding program by creating an F_2 cross between two phenotypically divergent outbred rat lines and then used this F_2 cross to map loci for the selection trait, sensation-seeking behavior, as well as several putatively correlated traits.

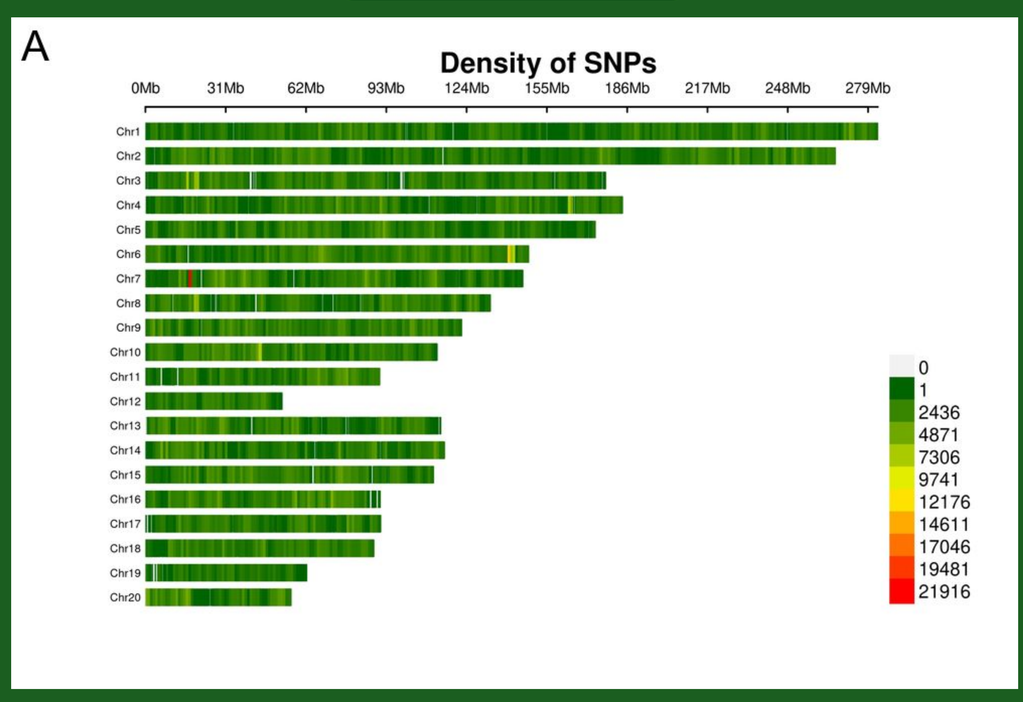
GENOTYPING

- We obtained 4,425,349 Single Nucleotide Polymorphisms (SNPs) across 538 F_2 rats using low-coverage whole genome sequencing (lcWGS).
- Multiplexed sequencing libraries were prepared using the Twist 96-Plex Library Preparation kit (and then sequenced on a NovaSeq 6000 platform (Illumina).
- To identify SNPs that segregate between bHR and bLR, we deeply sequenced 20 rats from the F_0 generation. We then used STITCH to impute genotypes in the F_2 using the SNPs from the 20 deeply sequenced F_0 rats as reference data.
- Next, we used BEAGLE to impute missing genotypes. To estimate genotyping accuracy and to identify potential sample mix-ups due to sample handling errors, we compared our genotypes to ~400 genotypes from an earlier microarray derived genotypes (Zhou et al., 2019).
- We used these dense, high-quality genotypes to perform genetic analysis.

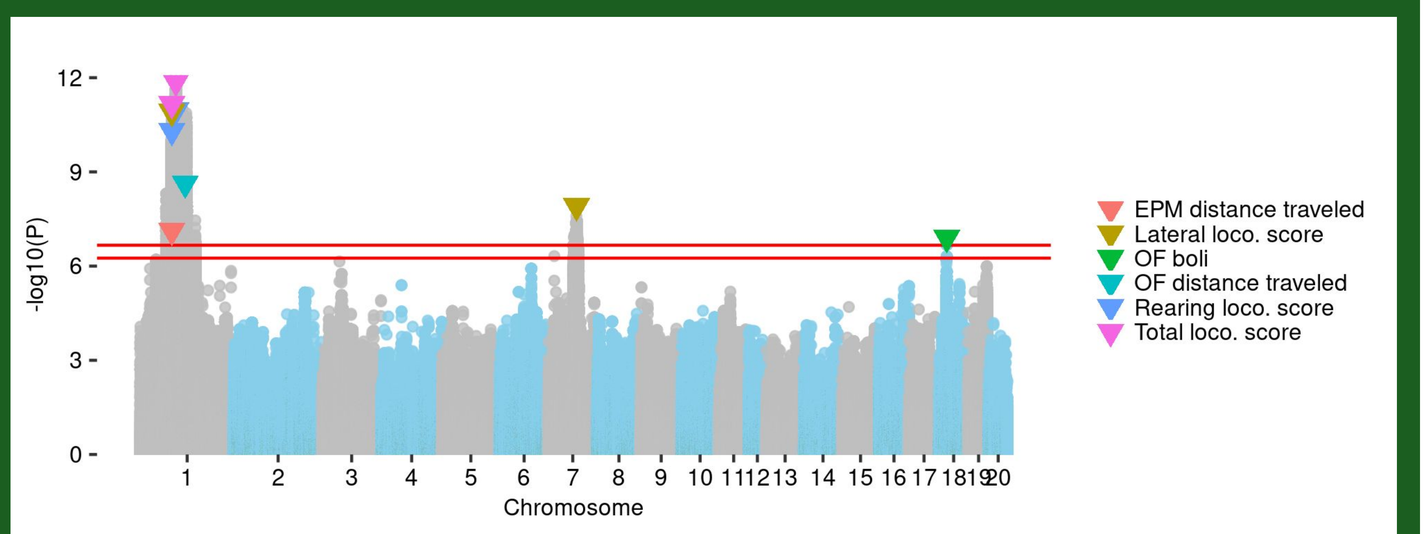
PHENOTYPING AND GWAS

- We phenotyped the animals for multiple behavioral traits.
- GWAS was performed using the MLMA-LOCO algorithm of GCTA software. SNP heritability estimates were also obtained with GCTA using the REML method.

Density of SNPs

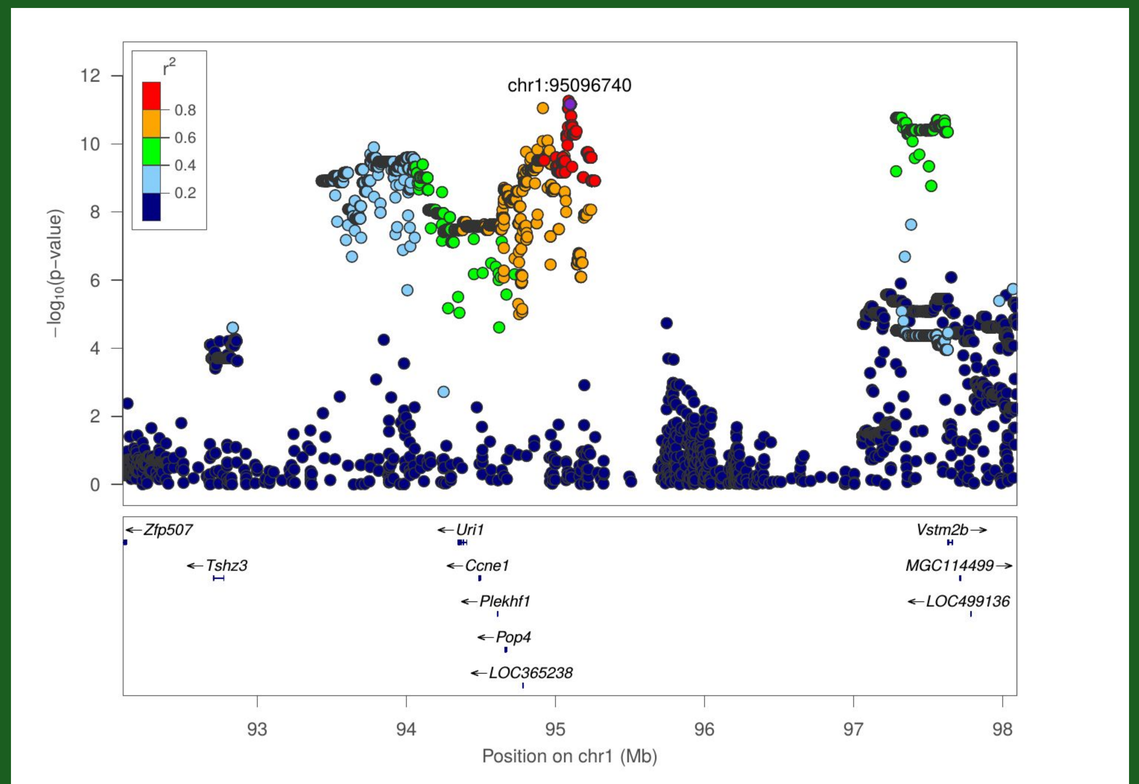


Porcupine plot for Locomotor score, EPM and OF traits.



The chromosomal distribution of all the P-values ($-\log_{10}(p)$ values) is shown, with top SNPs highlighted. The red lines show the threshold for significance. For EPM and Locomotor scores (i.e., LocoScore), the significance threshold was $-\log_{10}(p) = 6.664$ and for OF traits the threshold was $-\log_{10}(p) = 6.254$.

chr1:95 Mb QTL for Total locomotor score



Within the locus on chromosome 1 at about 95 Mb to which Exploratory Locomotion traits mapped, we identified a missense variant in the gene *Plekhf1*. This gene has previously been associated with glucocorticoid receptor signaling.

RESULTS

- The SNP heritability estimates for all the phenotypes ranged from 0.06 to 0.79. Lowest SNP h_2 estimates: PavCA measures and highest SNP h_2 estimates: Exploratory Locomotion traits.
- Despite the modest (for GWAS) sample size, we identified significant genetic associations for all facets of EL, OF, and EPM behaviors, but not for PavCA behavior.
- The loci were relatively small and contained several interesting genes.

CONCLUSIONS

- We discovered several genetic loci associated with complex behavior traits.
- Distance traveled measures from the open field and the elevated plus maze map onto different loci, thus may represent different aspects of novelty-induced locomotor activity.
- In conclusion, our selectively bred rat model reveals greater insight into the genetic architecture of sensation-seeking, anxiety, and addiction-related traits.

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