A 0.23 Mb region on mouse chromosome 11 contains three possible quantitative trait genes influencing methamphetamine sensitivity

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BACKGROUND: Sensitivity to the locomotor stimulant properties of drugs of abuse is mediated by shared neurocircuitry with drug reward; thus, determining its genetic basis will enhance our understanding of the neurobiology of addiction. We previously used mouse lines derived from C57BL/6J (B6) and DBA/2J (D2) strains that were selected for high and low sensitivity to methamphetamine (MA)-induced locomotor activity and identified a quantitative trait locus (QTL) on chromosome 11. In the present study, we phenotyped a genome-wide B6 x D2 F_2 cross and an F_8 advanced intercross to replicate this QTL and to fine map the locus. To further aid in identifying the quantitative trait gene(s) (QTGs) responsible for the QTL, we generated 17 subcongenic and sub-subcongenic lines carrying D2-derived intervals on a B6 background (B6.D2) spanning the entire chromosome 11.

METHODS: 676 F_2 mice and 552 F_8 mice were administered saline injections (10 ml/kg) on Days 1 and 2 in the open field (37.5 cm x 37.5 cm; AccuScan Instruments and Versamax software). On Day 3, mice were injected with MA (2 mg/kg, i.p.) and the total distance traveled was recorded over 30 min. Three B6.D2 congenic lines containing large D2-derived portions of chromosome 11 were originally obtained from Dr. Aldons Lusis's laboratory at UCLA (Lines 1, 6, and 9) and have since been backcrossed to B6 to introduce new recombination events and generate new lines. These subcongenic and sub-subcongenic lines were tested for MA sensitivity in an identical manner as described above and were always tested alongside wildtype littermate control mice. Congenic mice were genotyped using custom-made fluorescent markers from Applied Biosystems or PCR and traditional Sanger sequencing of genomic regions capturing B6/D2 SNPs. SNPs were chosen from the Mouse Phenome Database and the Sanger Institute SNP query website.

RESULTS: Genome-wide analysis of the F_2 cross, but not the F_8 cross, revealed a large, time-dependent QTL on chromosome 11 for MA-induced locomotor activity on Day 3 (peak LOD = 10) with a 1.5 LOD support interval of 30-70 Mb. The results of a congenic line (Line 1: 0-80 Mb) confirmed this QTL. Additionally, subcongenic lines spanning the region uncovered multiple, smaller-effect QTLs as evidenced by their different time-dependent effects on the time course for MA-induced locomotor activity and their different modes of inheritance. One particular subcongenic line (Line 4: 50-68 Mb) that flanked the peak marker of the F_2 study (55 Mb) captured a QTL that was confirmed via re-analysis of F_8 markers within this region. The latter observation indicated that the F_8 study was likely underpowered to reach significance for detecting this smaller-effect QTL. Next, we further dissected the 50-68 Mb locus by generating two sub-subcongenic lines that were congenic for a region spanning approximately 50-60 Mb. Owing to a fortuitous, proximal recombination event, these two lines possessed genotypes that differed only within a 0.23 Mb genomic region (50,186,508 Mb - 50,418,318 Mb; see Panel 7). The inheritance of the B6 allele within this small region was sufficient to reverse the phenotype from D2 to B6, demonstrating that at least one allele within this interval contributes to the QTL. There are only 3 annotated genes within the interval (*Hnrnph1*, *Rufy1*, and *Adamts2*). Thus, QTG identification is a tractable goal.

DISCUSSION: Using a combined approach of F_2, F_8 advanced intercross, and congenic analysis, we identified a 0.23 Mb region on chromosome 11 influencing MA-induced locomotor activity. According to the latest Sanger SNP dataset, there are at least two non-synonymous coding polymorphisms in *Rufy1*, indicating a potential functional consequence. *Rufy1* encodes for an early endosomal protein that is involved in trafficking following endocytosis, making this an intriguing candidate. Furthermore, there is a single SNP in the 5' UTR of *Hnrnph1* that could affect its expression. *Hnrnph1* encodes for a ribonuclear protein that binds RNAs and could influence pre-mRNA processing of a number of genes. We are currently conducting quantitative PCR experiments in congenic mice to determine if any of these three genes are differentially expressed - this would implicate the presence of *cis*-acting expression QTL(s) (eQTL) that regulate gene expression and behavior. Genomic analysis of gene expression will be used to identify potential gene networks modified by the 0.23 Mb region that may contribute to behavioral differences.

We have clear evidence that at least one gene within the 0.23 Mb region contributes to the QTL. However, it is possible that additional, distal D2 allele(s) outside of this region are also required to fully account for the effect on phenotype. Definitive evidence for the sole involvement of this region necessitates further backcrossing to replace the distal D2 alleles with B6 alleles. Furthermore, an important consideration is that there are several intergenic and intronic SNPs within the 0.23 Mb region that could exert functional genomic effects. Last, structural differences or omissions in the annotation could underlie the importance of this region.

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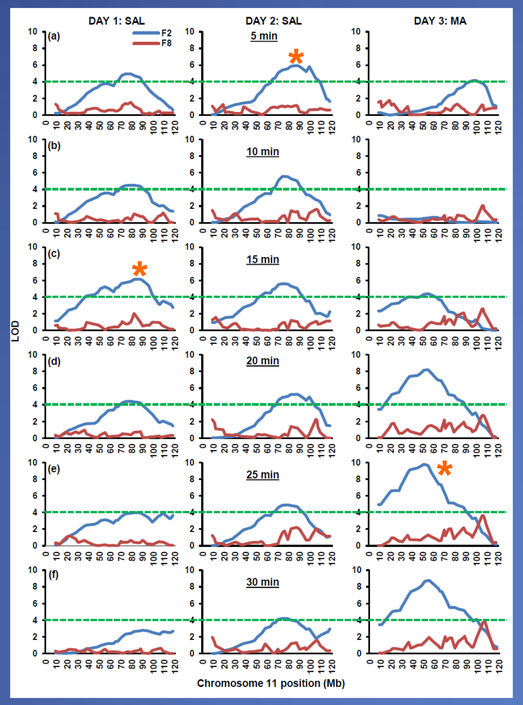
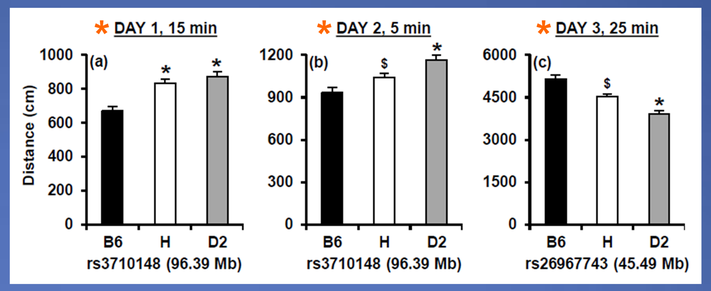
CONFLICTS OF INTEREST DISCLOSURES: The authors have no potential or perceived conflicts of interest to disclose.

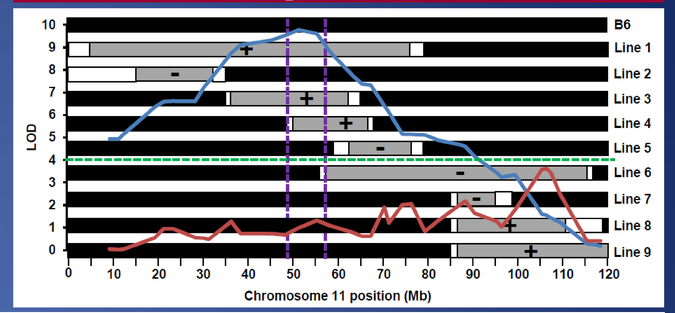
1. Time-dependent QTLs for locomotor activity on Days 1-3 in B6 x D2-F_2 and -F_8 mice



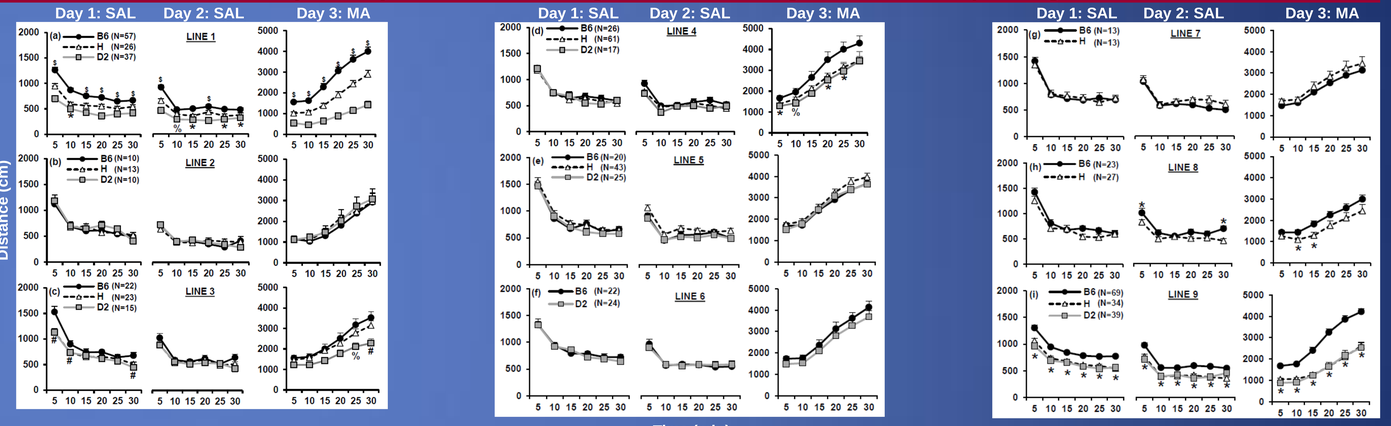
2. Effect plots for F_2 QTLs on Days 1-3 (peak time bins)



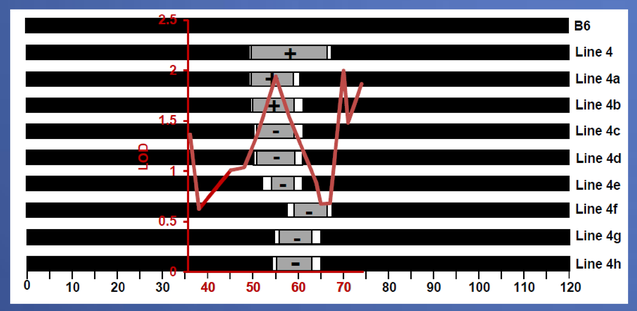
3. Co-mapping (+) of F_2 QTL for MA sensitivity in Line 4



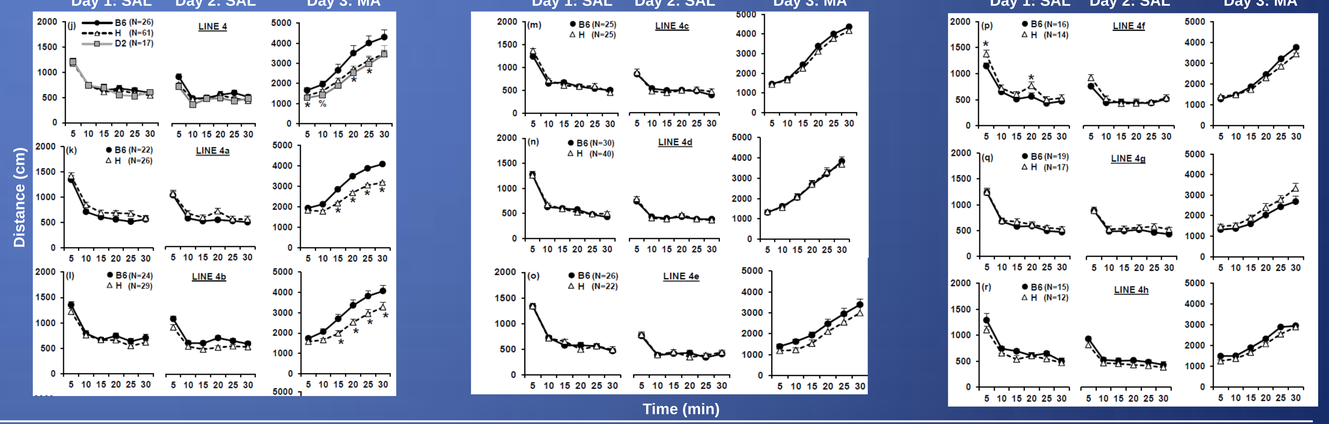
4. Locomotor activity for Days 1-3 in B6.D2 congenic and subcongenic Lines 1-9 spanning chromosome 11



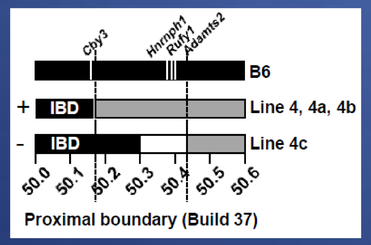
5. Co-mapping (+) of F_8 QTL for MA sensitivity in sub-subcongenics of Line 4



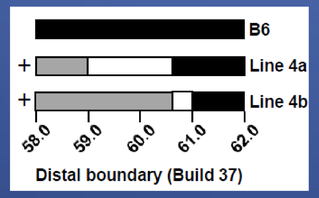
6. Locomotor activity for Days 1-3 in sub-subcongenic Lines 4a-4h



7. A recombination (Line 4b -> 4c) deduces the QTL to 0.23 Mb



8. Distal breakpoints in Lines 4a (+) and 4b (+) demonstrate independent QTL replication



9. Rufy1/Rabip4 is a candidate gene for methamphetamine sensitivity

- Rufy1/Rabip4 interacts with Rab4.
- Rab4 translocates to the plasma membrane during dopamine release (Kost et al., 2011)
- Rab4 is involved in constitutive recycling of DAT and D2 receptors (Eriksen et al., 2010; Li et al., 2012).
- Rab4 contributes to amphetamine-induced endocytosis of NET (Matthies et al., 2010).

10. Summary and Conclusions

- F_2, F_8, and B6.D2 congenic analysis revealed multiple large and small-effect QTLs for MA sensitivity on chromosome 11.
- We pursued a dominantly transmitted congenic QTL and identified a 0.23 Mb critical interval containing just three possible genes.
- ***Rufy1*** is a novel candidate gene for MA sensitivity. It contains two B6/D2 coding polymorphisms; these could produce functional effects on MA-induced transporter trafficking and/or DA-induced receptor trafficking.
- Deep congenic analysis yielded near-QTG resolution and novel hypotheses regarding neurobiological mechanisms of MA behavior.