A 0.23 Mb region regulates methamphetamine sensitivity in mice

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BACKGROUND: Sensitivity to the locomotor stimulant properties of drugs of abuse is heritable and its genetic basis may be shared with alleles that modulate drug reward. Therefore, identifying novel genetic factors for this trait will enhance our understanding of the neurobiological basis of addiction. More than 10 years ago, we selectively bred mice for high and low methamphetamine (MA) sensitivity from a C57BL/6J (B6) x DBA/2J (D2) cross and identified a quantitative trait locus (QTL) on chromosome 11. Here, we utilized the power of an F_2 cross and the iterative nature of interval-specific congenic lines to replicate and fine map this QTL.

METHODS: 676 F_2 mice were administered saline injections (10 ml/kg, i.p.) on Days 1 and 2 in the open field (37.5 cm x 37.5 cm). On Day 3, mice were injected with MA (2 mg/kg, i.p.) and the total distance traveled was recorded over 30 min. Congenic lines containing large D2-derived portions of chromosome 11 were obtained from Dr. Aldons Lusis's laboratory at UCLA and backcrossed to B6 to generate new recombination events. These new lines were tested alongside wildtype littermates. Congenic mice were genotyped using custom-designed fluorescent markers or PCR and traditional Sanger sequencing of genomic regions that contained SNPs. Data were analyzed using interval mapping in R (QTLRel) or repeated measures ANOVA with genotype as the factor and time as the repeated measure.

RESULTS: We replicated a large-effect QTL in F_2 mice on chromosome 11 (50 Mb; LOD = 10; 1.5 LOD-support interval = 30-70 Mb) that was specific for MA-induced locomotor activity and was confirmed in a congenic line (0-80 Mb). The results of subcongenic lines revealed multiple, smaller-effect loci with different modes of inheritance. One QTL converged remarkably well with the F_2 peak at 50 Mb – thus, we aggressively pursued this locus via repeated backcrossing to the parental B6 strain. Owing to a fortuitous recombination event, we identified and replicated a critical interval (50,190,790-50,400,235 bp; Build 37) that contains only two protein-coding genes (*Hnrnph1*, *Rufy1*) and one pseudogene (Gm12197). Inheritance of the B6 allele within this interval completely reversed the phenotype from D2 to B6. None of the protein-coding genes were differentially expressed in the striatum or cortex. *Rufy1* is the only gene that contains any nonsynonymous coding polymorphisms and we confirmed all three of these SNPs via Sanger sequencing.

DISCUSSION: We identified a 0.21 Mb region on chromosome 11 that influenced MA-induced locomotor activity. The region contains two protein coding genes: *Rufy1* and *Hnrnph1*. Neither of these genes were differentially expressed in the striatum or cortex (data not shown), suggesting that a missense SNP within the critical interval could be responsible for the QTL. *Rufy1* contains three missense SNPs and codes for an endosomal protein that interacts with Rab and could affect MA-induced trafficking of monoamine transporters (the primary molecular targets of amphetamines). Interestingly, a *RUFY1* polymorphism in humans was associated with the endophenotype "body dissatisfaction" in patients with eating disorders (Boraska et al., 2012). It is worthy to note that amphetamines are the most efficacious drug class for appetite suppression and are misused precisely for this purpose by patients with eating disorders. Thus, *RUFY1* could represent a common genetic mechanism for sensitivity to amphetamines and endophenotypes associated with eating disorders.

We utilized the congenic approach to QTL mapping by maximally leveraging our focus on a single QTL where we identified and replicated a 0.21 Mb interval for a complex trait. This is an extremely rare feat for genetic mapping studies in mice. Following nearly six years of backcrossing, phenotyping, diligence, and fortuity, we have derived a limited number of hypotheses regarding the genes and mechanisms by which this QTL regulates MA sensitivity. This level of resolution and experimental validation was made possible only by the knowledge we have from bioinformatic resources in next-generation DNA re-sequencing of inbred mouse strains and expression QTLs. Future studies involving successive gene targeting and functional characterization will define this novel genetic and neurobiological mechanism of action.

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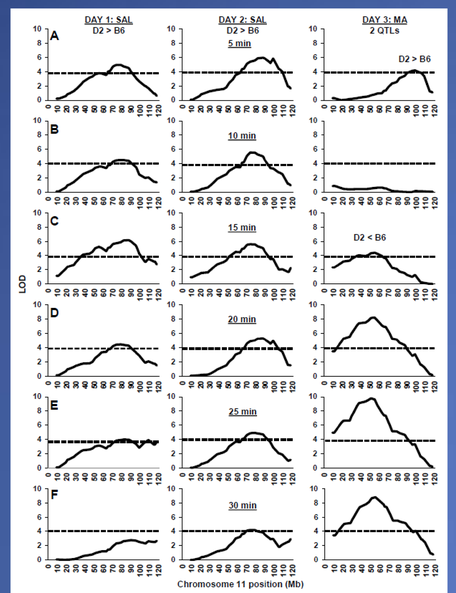
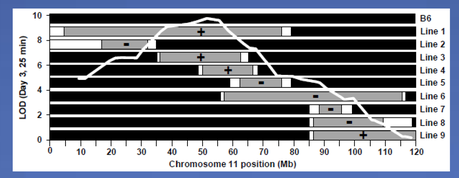
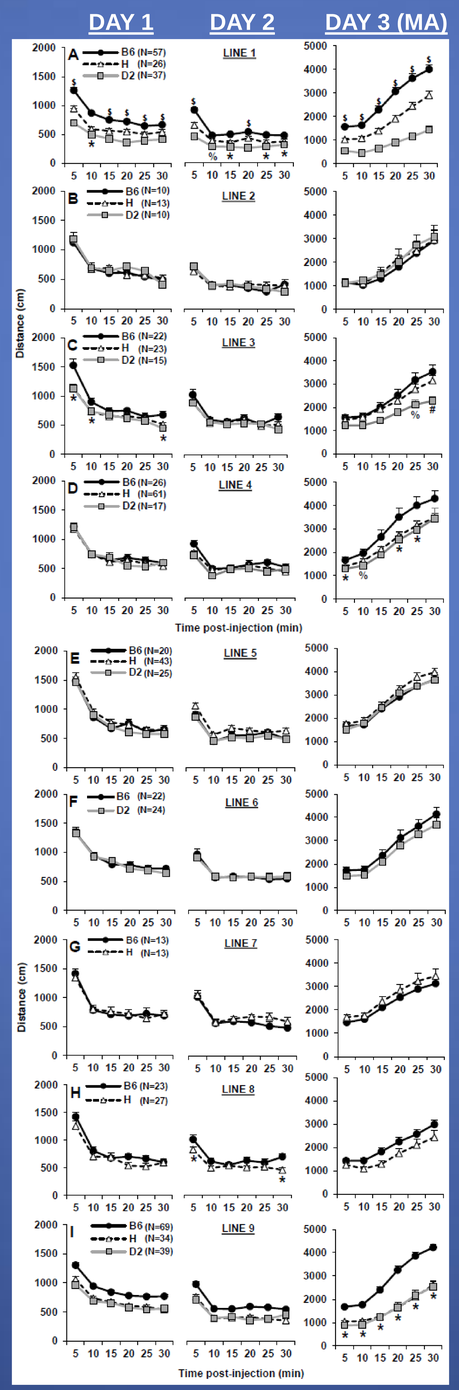
1. QTL for methamphetamine (MA) sensitivity in B6 x D2-F_2 mice

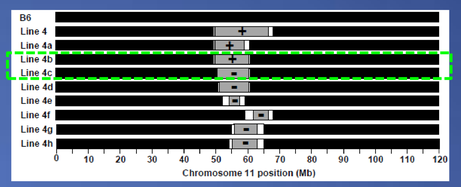


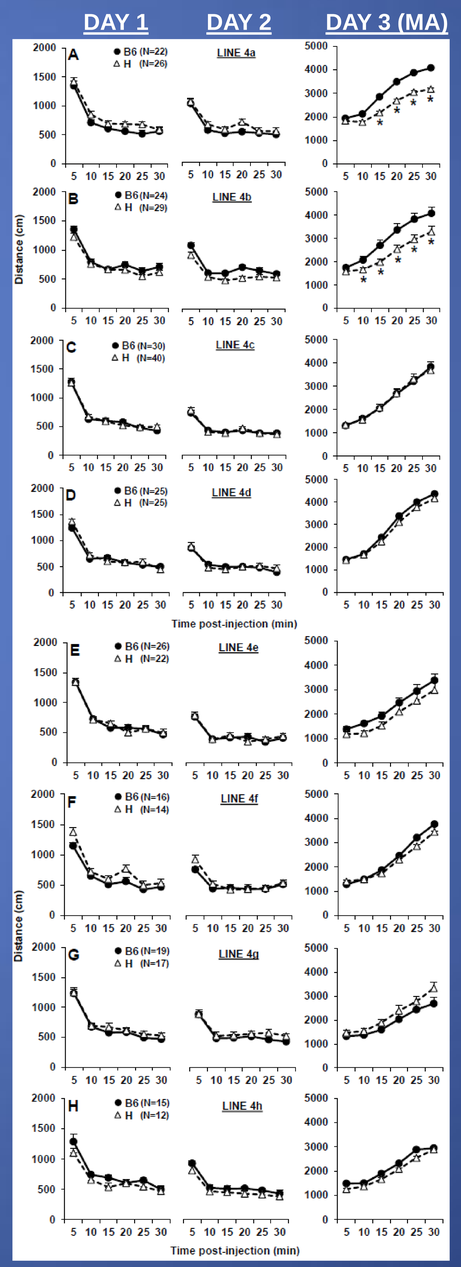
2. Co-mapping the QTL (+) in F_2 and congenic lines



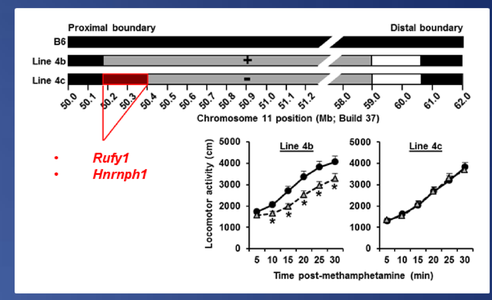


3. Fine mapping the QTL (+) in subcongenic lines





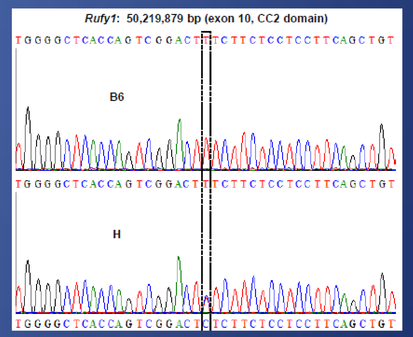
4. A fortuitous recombination event in Line 4c defines the critical interval



5. Genetic variants within critical interval *(http://www.sanger.ac.uk/)*

Build 37	Chr. 11 bp)	NC transc variant	NC exon variant	US gene variant	DS gene variant	Intron variant	Non-synon	synon	Intergenic variant
Hnrnph1	50190596-50200030	1	2	0	1	7	0	0	-
Intergenic1	50200031-50202804	-	-	-	-	-	-	-	-
Rufy1	50202805-50244613	104	0	31	0	165	3	1	-
Intergenic2	50244614-50260078	-	-	-	-	-	-	-	12
Gm12197	50260079-50260525	0	0	5	11	0	0	0	-
Intergenic3	50260526-50400235	-	-	-	-	-	-	-	253

6. Missense SNP in exon 10 of the CC2 domain of *Rufy1*



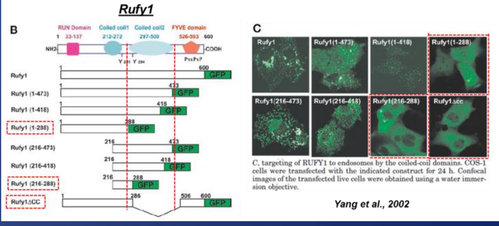
7. *Rufy1* is a candidate gene for MA sensitivity

- Neither *Hnrnph1* nor *Rufy1* was differentially expressed in the striatum or cortex (data not shown).
- *Rufy1* possesses 3 missense SNPs. One is located in the CC2 domain which is crucial for endosome localization.
- *Rufy1* interacts with Rab proteins.
- Rab proteins contribute to constitutive and amphetamine-induced trafficking of monoamine transporters (*Matthies et al., 2010*).
- We hypothesize that one or more mutations in *Rufy1* affect MA-induced trafficking of its primary molecular target – the dopamine transporter.

8. Summary and future directions

- We identified a 0.21 Mb critical interval for MA sensitivity that contains just two protein coding genes: *Rufy1 and Hnrnph1*
- Select gene targeting of both *Rufy1* and *Hnrnph1* is underway and will aid in quantitative trait gene identification.
- Transcriptome analysis via RNA-sequencing is underway and will identify potential gene networks modulated by alleles within the locus.

CC2 domain is necessary for targeting of *Rufy1* to the endosome



Yang J, Kim O, Wu J, Qiu Y (2002). Interaction between tyrosine Etk and RUN domain- and FYVE domain-containing protein RUFY1. A possible role of ETK regulation of vesicle trafficking. *J Biol Chem. Aug 16;277(33):30219-26*