Systems genetic analysis and positional cloning in a reduced complexity cross identifies a major QTL on distal chromosome 1 underlying opioid addiction traits

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BACKGROUND: Opioid addiction is a national epidemic. Both genetic and non-genetic factors contribute to opioid addiction; however, the genetic basis remains largely unknown. Furthermore, treatments are limited primarily to opioid maintenance therapy. A better understanding of the genetic and non-genetic factors influencing opioid addiction could lead to improved therapeutics that promote cessation of opioid use. Mice are a powerful mammalian model organism for genetic discovery of complex diseases. Quantitative trait locus (QTL) mapping is an unbiased, discovery-based approach to identifying the genetic basis of disease-relevant traits, including behavioral addiction traits. Two main challenges in murine QTL analysis include overcoming genetic complexity and resolving QTL intervals to a tractable number of candidate genes, both of which are necessary to facilitate validation of functional genes and variants. We developed a strategy to overcome these challenges using a Reduced Complexity Cross (RCC) between closely related C57BL/6 substrains combined with immediate fine mapping in F2 recombinants. Additionally, we used transcriptome and expression QTL (eQTL) analysis via mRNA sequencing (RNA-seq) to identify candidate genes and neurobiological mechanisms underlying opioid addiction traits.

METHODS: We mapped the genetic basis of opioid addiction traits in a cross between very closely related substrains of C57BL/6 mice – the Reduced Complexity Cross. We phenotyped 213 saline (SAL)-, 212 oxycodone (OXY)-, and 209 naloxone (NAL)-trained mice for locomotor activity, conditioned drug reward/aversion, and state-dependent drug reward/aversion. Additionally, we continued to treat a subset of SAL and OXY mice for an additional two weeks where we assessed OXY analgesic tolerance and dependence as measured via analgesia on the hot plate (52.5ºC) and emotional-affective withdrawal on the elevated plus maze (EPM). 96 makers on a custom Fluidigm array were used for genotyping, with an average spacing of 15 cM between each adjacent marker. QTL mapping was conducted in R/qtl using Haley-Knott regression, with 1000 permutations to determine significance threshold and Bayes credible interval to determine the QTL coordinates. Differential gene expression was conducted in a subset of F2 mice capturing a QTL for OXY-induced locomotor activity and withdrawal (n=23). RNA-seq was conducted using Illumina technologies (100 bp, paired end reads, poly-A selected, average of 70 million reads per sample) The Bioconductor package *limma* was used to conduct transcriptome analysis (FDR<5%). Additionally, eQTL and exon-level expression QTL (eeQTL) analysis were conducted in MatrixEQTL (p<0.01). Fine mapping via positional cloning was conducted for a single Mendelian QTL underlying OXY-induced locomotor activity and withdrawal by selectively choosing F2 recombinants within the QTL interval, backcrossing, and phenotyping at each generation. Because the trait exhibited Mendelian inheritance, we could ignore background genetic variation outside of the QTL interval and focus solely on recombination events within the QTL interval and assessing their effect on behavior. Enrichment analysis of biological pathways and gene networks was conducted using Enrichr and Ingenuity Pathway Analysis. (IPA).

RESULTS: We mapped a single genome-wide significant QTL influencing OXY-induced locomotor traits and emotional-affective withdrawal to distal chromosome 1 (LOD=8.9-13.2; 171-181 Mb and LOD=4.7; 152-180 Mb, respectively). Fine mapping via backcrossing selectively chosen recombinant F2 mice and phenotyping for several consecutive generations effectively reduced the size of the QTL interval from 28 Mb to 6 Mb (chr. 1: 167.7-173.7 Mb). There were 12 eQTL genes within 2 Mb of the the 6 Mb interval, including *Gpr161, Pou2f1, Ildr2, Pcp4l1, Ncstn, Atp1a2, Kcnj9, Igsf9, Cadm3, Aim2, and Rgs7.* We validated differential expression of *Aim2* and *Cadm3* at the protein level in the B6 parental substrains, providing functional support for their candidacy as quantitative trait genes underlying opioid addiction traits. Finally, IPA identified a network of genes associated with the chr. 1 QTL genotype and chronic OXY treatment that contained two hub genes that are well-known to be involved in in neurodegenerative disease, including amyloid precursor protein (APP) and microtubule-associated protein tau (MAPT).

CONCLUSION: Systems genetic and fine mapping in the RCC led quickly to the identification of QTLs, high-confidence, candidate QTL genes, and downstream biological pathways underlying opioid addiction traits. Further positional cloning and gene editing will identify the quantitative trait gene and functional variant. These results provide new insight into the genetic and neurobiological bases of opioid addiction and will inform human translational genetics and therapeutics.

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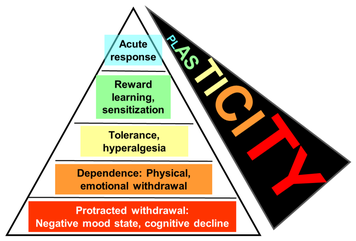
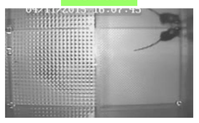
#1: Multi-stage addiction assessment protocol (**MSAAP**) in a Reduced Complexity Cross (**RCC**) between C57BL/6J & C57BL/6NJ

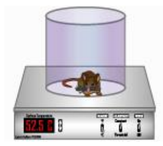
Multistage addiction assessment protocol (MSAAP)	OXY Regimen	Behavioral assay	Plasticity (time)
Acute (locomotor, analgesia)	1.25 mg/kg (1X)	Locomotor	Min, hrs
Reward conditioning	1.25 mg/kg (2X)	Conditioned place preference	Days
Analgesic tolerance	20 mg/kg (4X)	Analgesia	Week(s)
Dependence: Spontaneous withdrawal	20 mg/kg (4X)	Emotional-affective	Weeks
Abstinence	Drug-free	_____	Weeks
Protracted withdrawal	Drug-free	Anxiety-like, cognitive	Months



Locomotor
Reward

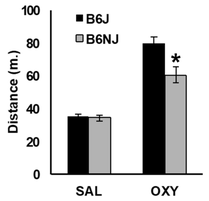


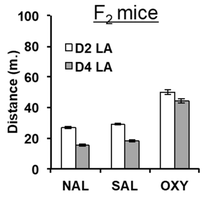
Tolerance

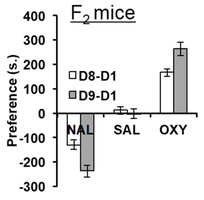


Spontaneous withdrawal (EPM)

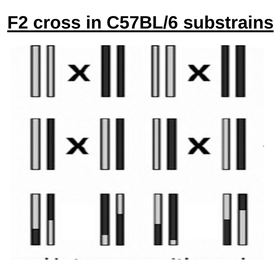
Day 2: Acute



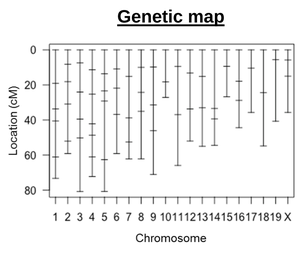




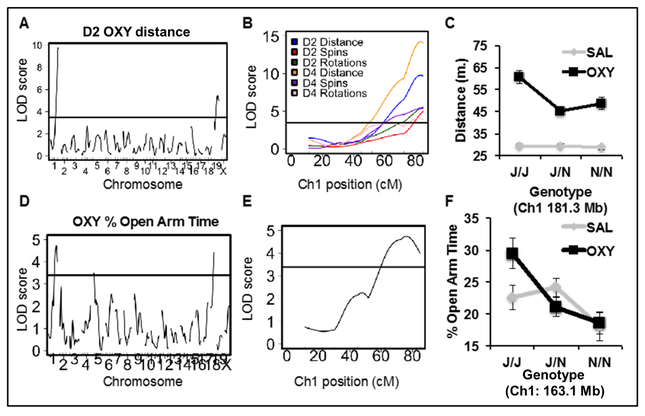
F2 cross in C57BL/6 substrains



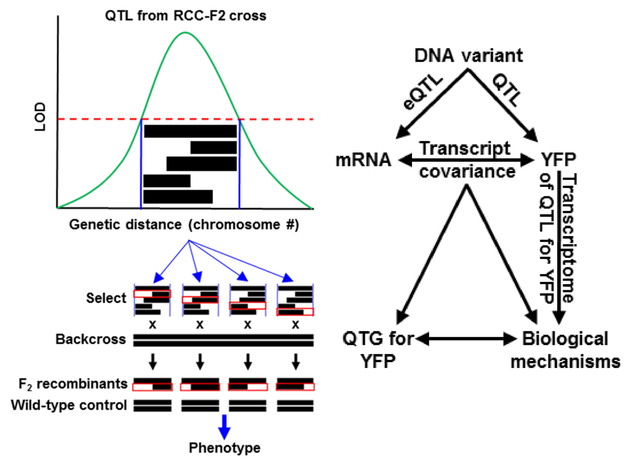
Genetic map



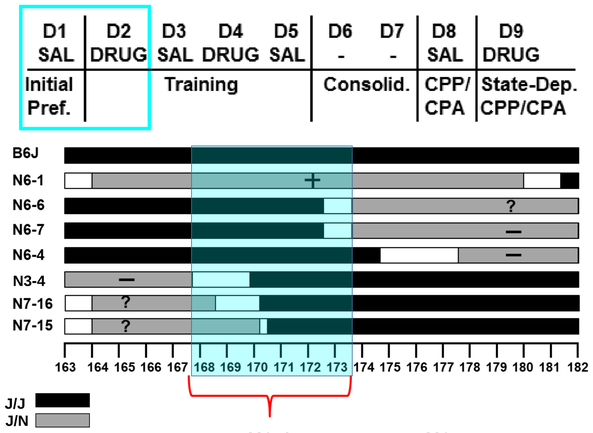
#2: A major QTL on distal chromosome 1 influences OXY-induced locomotor activity and spontaneous emotional withdrawal



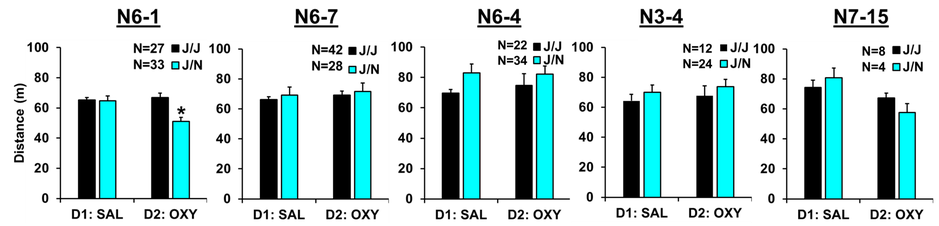
#3: Fine mapping and systems genetic analysis of opioid addiction traits in the RCC



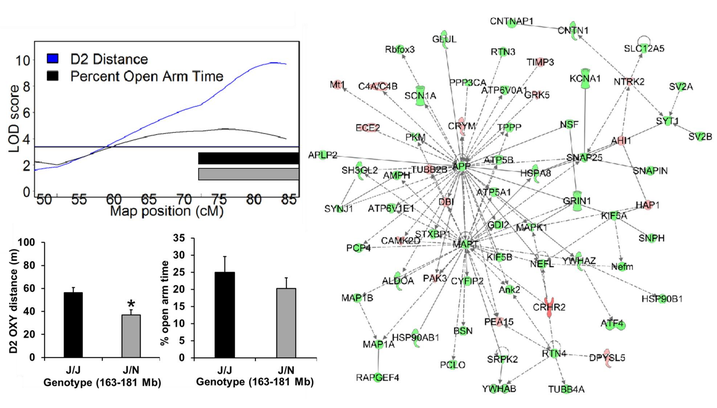
#4: Fine mapping of a 6 Mb region on chr. 1 influencing acute OXY induced locomotor activity and genes containing SNPs within this 167.7-173.7 Mb interval

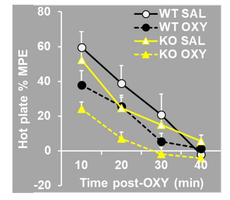


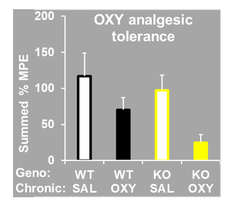
Gene	Position (Mb)	Gene	Position (Mb)	Gene	Position (Mb)
Lmx1a	167.7	Gm27844	171.1	Slamf1	171.8
Gm20711	168.1	1700009P17Rik	171.1	Cd84	171.8
Pbx1	168.1	Apoa2	171.2	RP23-77A8.2	171.8
RP23-167H24.1	169.4	Fcer1g	171.2	Slamf6	171.9
RP24-570C24.2	169.4	Ndufs2	171.2	Vangl2	172.0
Rgs5	169.7	Adamts4	171.3	Nhlh1	172.0
Gm22114	169.8	B4galt3	171.3	Pex19	172.1
1700084C01Rik	169.9	Ppox	171.3	Dcaf8	172.2
Ddr2	170.0	Usp21	171.3	Casq1	172.2
Uap1	170.2	Dedd	171.3	Atp1a4	172.2
Uhmk1	170.2	Nit1	171.3	Igsf8	172.3
Gm7694	170.3	Pfdn2	171.3	RP24-144H23.4	172.5
Nos1ap	170.4	Pvrl4	171.4	Slamf9	172.5
Olfml2b	170.7	Arhgap30	171.4	Cfap45	172.5
Atf6	170.7	Gm26641	171.4	Slamf8	172.6
Dusp12	170.9	B930036N10Rik	171.5	Dusp23	172.6
RP24-65D16.2	170.9	Itln1	171.5	Gm10075	172.7
Fcgr2b	171.0	Cd244	171.6	Crp	172.7
RP24-65D16.4	171.0	Slamf7	171.7	Olfr1409-ps1	173.1
Fcgr4	171.0	RP23-145F9.4	171.7	Cadm3	173.3
Fcgr3	171.1	Cd48	171.7	Aim2	173.4
Gm27552	171.1	Tma7-ps	171.7	Pydc3	173.7

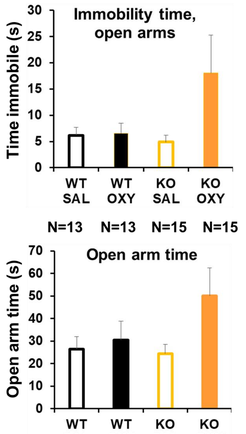


#5: Striatal transcriptomics of the chr. 1 QTL in OXY mice identifies *App* and *Mapt* as hub genes: Validation of ***Mapt* (*tau*) $^{-/-}$ mice** in OXY tolerance and dependence









Mapt $^{-/-}$ *mice were bred in Ben Wolozin's lab*

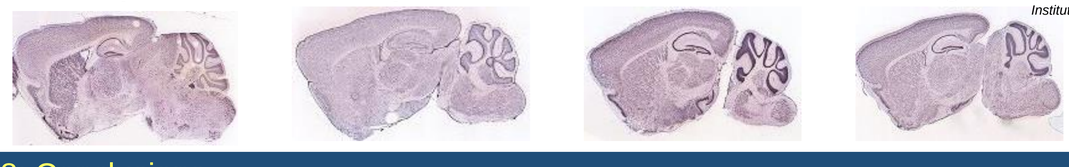
#6: Striatal eQTL genes within 2 Mb of the distal chr. 1 interval (167.7 Mb-173.7 Mb)

eQTL gene	Gene Name	chr	Mb	SNP (Mb)	Exp.	P. Assoc.	Adj. P	SNPs?	Reg. D2	r, D2	Reg. D4	r, D4	Reg. EPM	r, EPM
Gpr161	G protein-coupled receptor 161	1	165.3	181.3	3.5	1.4E-03	1.7E-02	no	___	___	___	___	___	___
Pou2f1	POU domain, class 2, transcription factor 1	1	165.9	181.3	5.37	2.2E-06	1.4E-04	no	9.9E-03	___	___	___	___	___
Ildr2	immunoglobulin-like domain containing receptor 2	1	166.3	181.3	6.66	7.0E-04	1.0E-02	no	4.2E-04	0.42	3.4E-02	___	1.1E-07	0.55
Nuf2	NUF2, NDC80 kinetochore complex component	1	169.5	181.3	1.53	3.2E-03	3.1E-02	no	___	___	___	___	___	___
Pcp4l1	**Purkinje cell protein 4-like 1**	**1**	**171.2**	**181.3**	**8.64**	**4.2E-06**	**2.3E-04**	**no**	**5.3 E-09**	**-0.53**	**3.4 E-07**	**-0.48**	**1.6E-08**	**-0.55**
Ncstn	nicastrin	1	172.1	181.3	5.72	6.8E-04	1.0E-02	no	___	___	___	___	___	___
Atp1a2	**ATPase, Na+/K+ transporting, alpha 2 polypeptide**	**1**	**172.3**	**181.3**	**9.74**	**9.6E-05**	**2.3E-03**	**no**	**2.2 E-05**	**0.37**	**3.5 E-07**	**0.45**	**1.0E-02**	___
Kcnj9	K+ inwardly-rectifying channel, subfamily J, member 9	1	172.3	181.3	6.74	1.1E-03	1.4E-02	no	3.9E-03	___	___	___	4.2E-04	0.44
Igsf9	**immunoglobulin superfamily, member 9**	**1**	**172.5**	**181.3**	**3.78**	**2.4E-03**	**2.5E-02**	**no**	**3.5E-04**	**-0.48**	**9.5E-04**	**-0.46**	**6.9E-04**	**-0.46**
Cadm3	**cell adhesion molecule 3**	1	173.3	181.3	8.63	2.1E-05	7.5E-04	**yes**	___	___	___	___	2.5E-02	___
Aim2	**absent in melanoma 2**	1	173.4	181.3	1.84	2.0E-03	2.2E-02	**yes**	___	___	___	___	___	___
Rgs7	**Regulator of g-protein signaling 7**	1	175.5	181.3	4.93	1.5E-03	1.8E-02	**yes**	1.4E-02	0.58	___	0.35	1.5E-02	___

#7: *Pcpl1* and *Atp1a2* are top positional and functional candidate genes underlying OXY addiction traits

Pcp4l ***Atp1a2*** ***Cadm3*** ***Rgs7***

Images courtesy of the Allen Brain Institute



#8: Conclusions

- QTL mapping of opioid addiction traits identified a major locus on distal chr. 1 (160-180 Mb).
- Rapid fine mapping via select backcrossing of F2 recombinants reduced the size of the interval to 6 Mb.
- Network analysis of the QTL identified an App-Mapt dual hub network that we are validating for opioid addiction traits.
- Striatal eQTL analysis and transcript-behavior regression analysis prioritized candidate genes.
- Continued fine mapping and gene editing will identify the positional and functional quantitative trait gene(s).