From biomarkers to drug targets: genetic animal models of stress and psychiatric disorders

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GENE or ENVIRONMENT?

- Complex, but even single-gene diseases have genetic and environmental contribution to individual variation

- Complex diseases affect the most people
- Environment is everything that is not heritable, but what about epigenetics?!?!

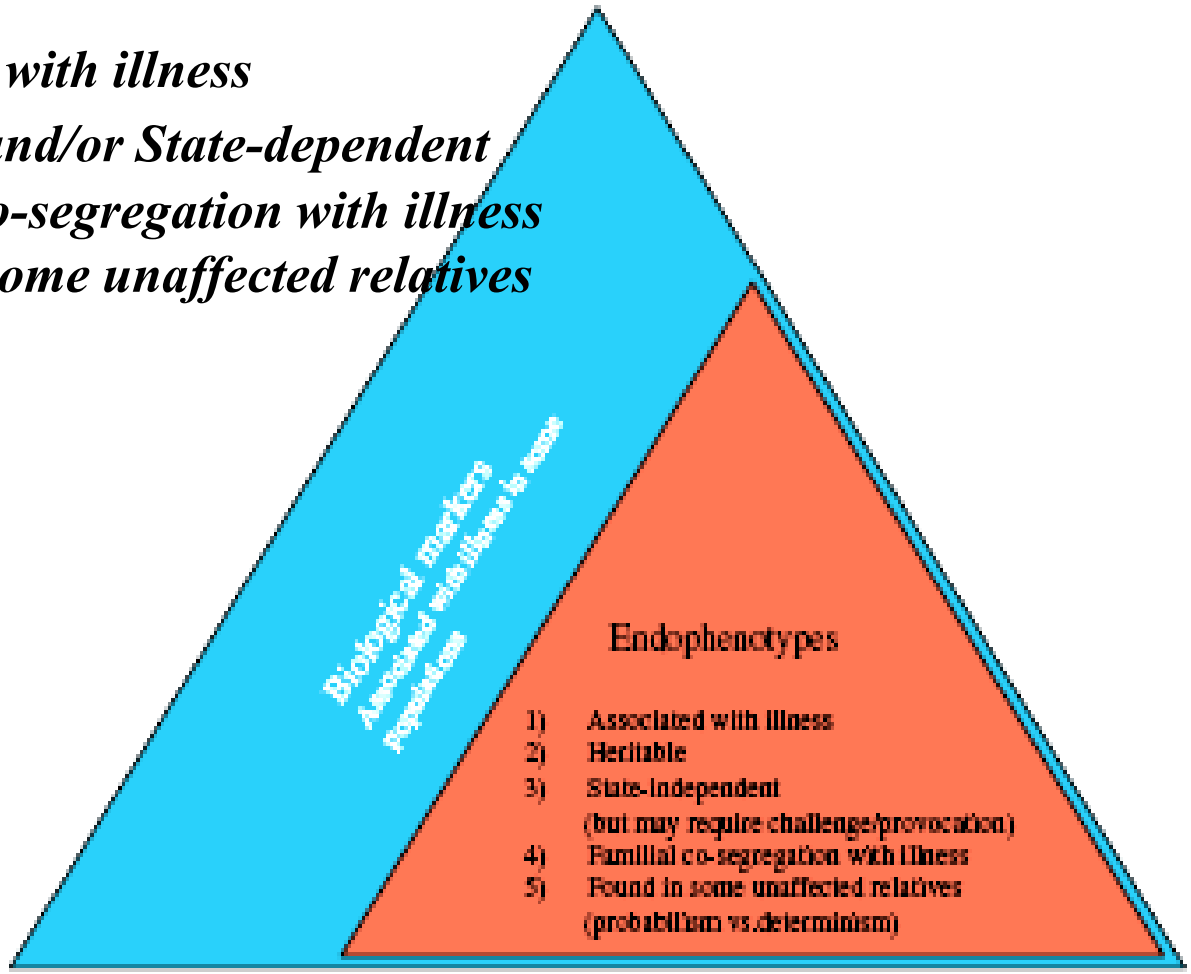
Animal Models to Study Human Diseases

No single animal model can fully replicate any human disorder. Each model, however, can help to clarify certain aspects of the disorder (the concept of endophenotypes).

Biological markers: associated with illness in some population

Endophenotypes are specific manifestation of an illness:

- *Associated with illness*
- *Heritable and/or State-dependent*
- *Familial co-segregation with illness*
- *Found in some unaffected relatives*



Genome-wide association studies, and why animal models are still useful

- **GWAS can**
- successfully localize positive signal to a few tens of kilobases, usually the span of a single gene, therefore,
- identify the genetic determinants of human complex disease directly

- But,
- In most diseases, genetic variation segregating at these genes explains only a small fraction of cases that could be accounted for by genetic causes
- Environment cannot be controlled in humans
- Function of the gene(s) can be elucidated in animal models

Outline

- Genetic contribution to individual coping with stress
  - Quantitative Trait Loci analysis
  - X-congenic strains
  - Genetic and transcriptomics: a combined analysis
- Genetic contribution to depressive behavior
  - Quantitative Trait Loci analysis
  - Genetic and transcriptomics: a combined analysis
  - Biomarkers for depression: a translational approach

What is Coping?

- Definition 1: The cognitive, behavioral and physiological processes aimed at diminishing or terminating **stress**.

- Definition 2: General **adaptive** patterns of responses to environmental challenges

Coping Styles are:

- Stable trait characteristics: active and passive coping strategies

- Genetic contribution: higher concordance in identical twins; heritable in animals

Implications for health and disease:

- **Passive coping increases vulnerability to**
  - **Heart disease**
  - **Hypertension**
  - **Diabetes**
  - **Rheumatoid arthritis pain (perception)**
  - **Anxiety disorders**
  - **Depressive disorders, etc.**
- **Active coping increases resilience in the face of adversity**

How do we measure coping strategies?

Any behavioral test with a choice for active or passive response to a threat, without punishment!

DB (Defensive Burying) a test with ethological validity

- The animal can avoid the threat by actively burying the prod or passively avoiding it.
- Approaching the prod is a form of stereotypy

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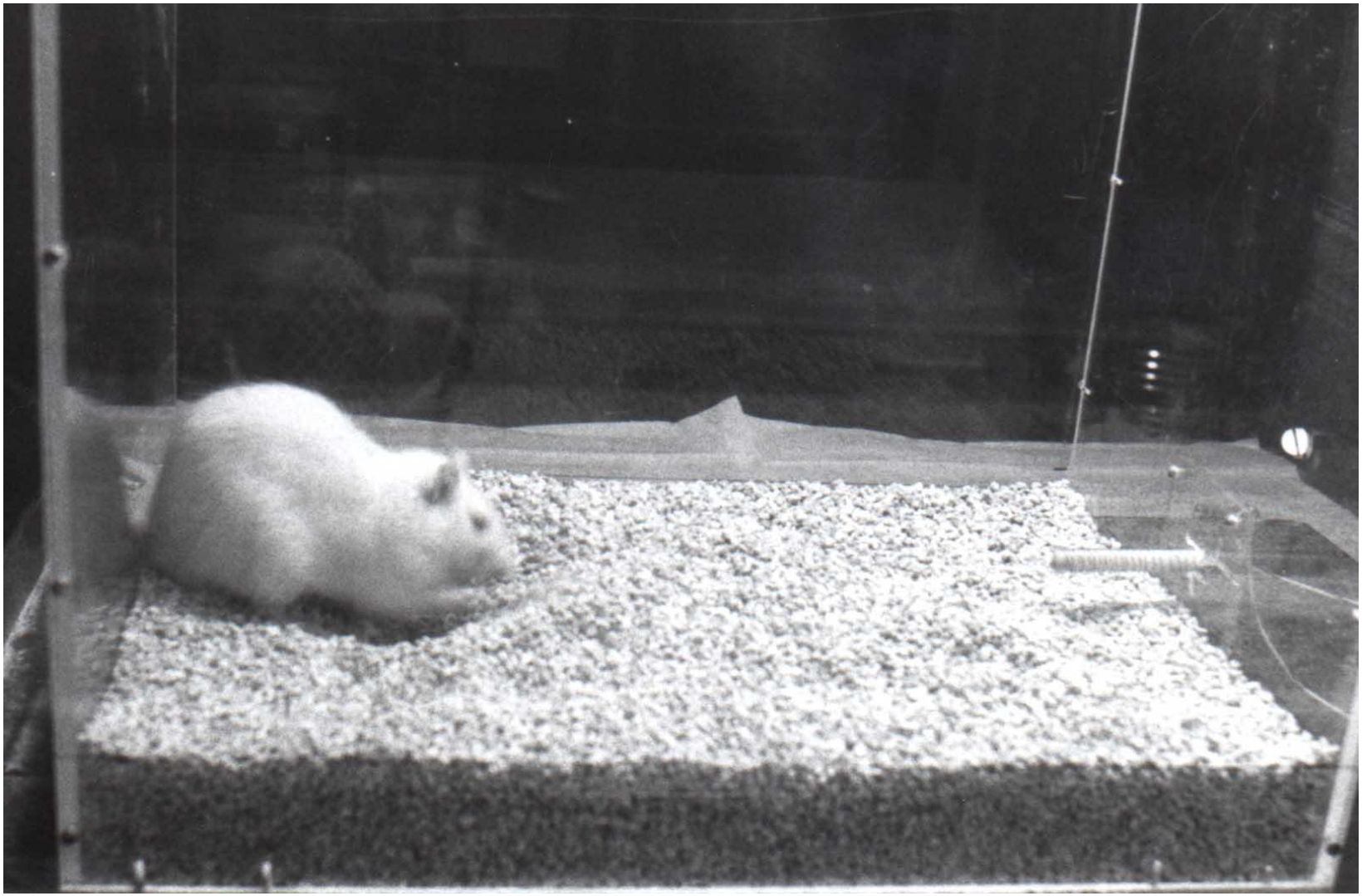

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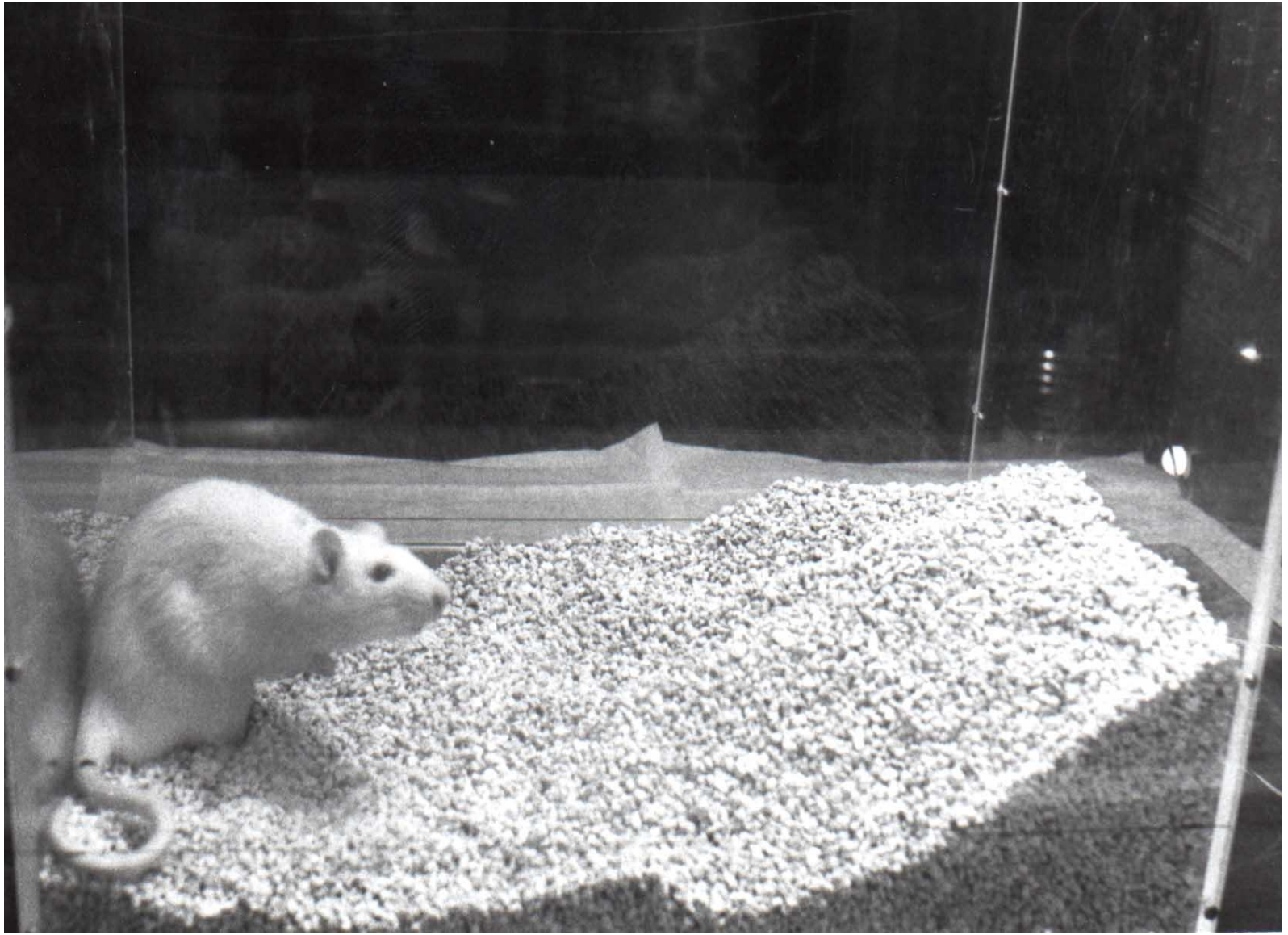

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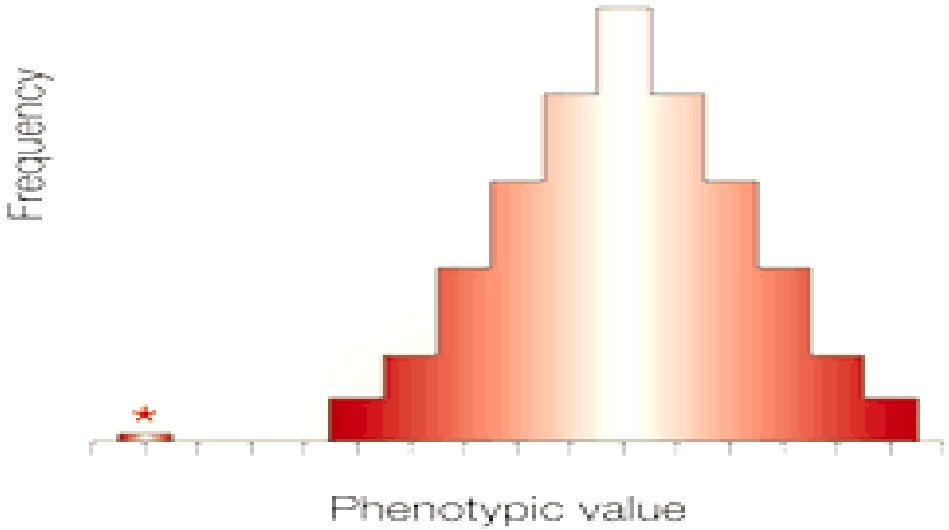
Strategy to identify Quantitative Trait Genes

- QTL analyses identify chromosomal location
- Congenic strains are generated
- Congenic strains are characterized behaviorally
- SNP analysis of parental strains compared to reference strain BN narrow regions
- Microarray analyses of relevant brain regions identify differentially expressed genes within these narrow regions
- These genes are candidate QTGs waiting functional confirmation

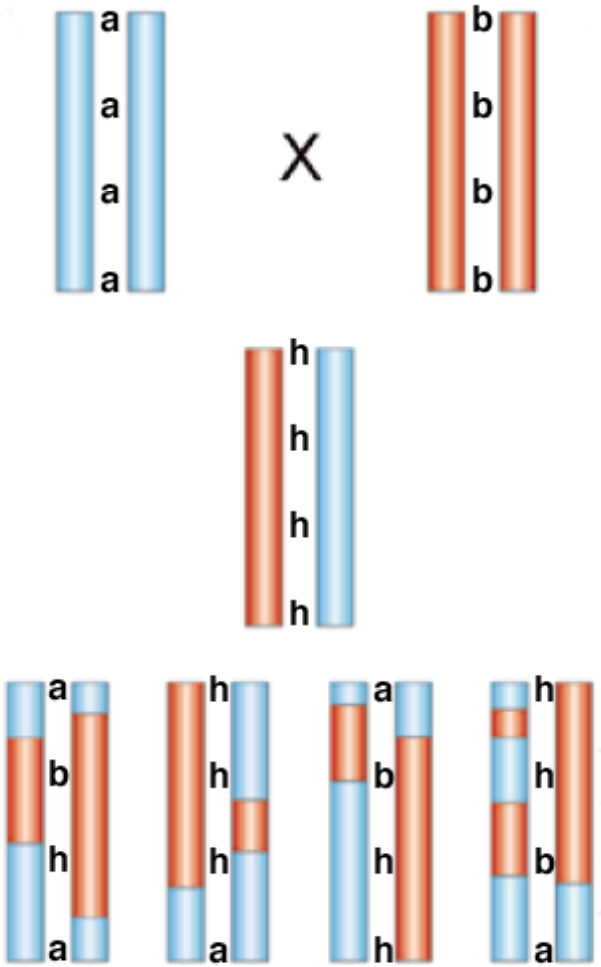
Quantitative Trait Loci Analysis (QTL)

Quantitative Trait – a heritable character that is transmitted as continuous variation

Loci - positions that genes occupy on a chromosome



Quantitative Trait Locus Analysis



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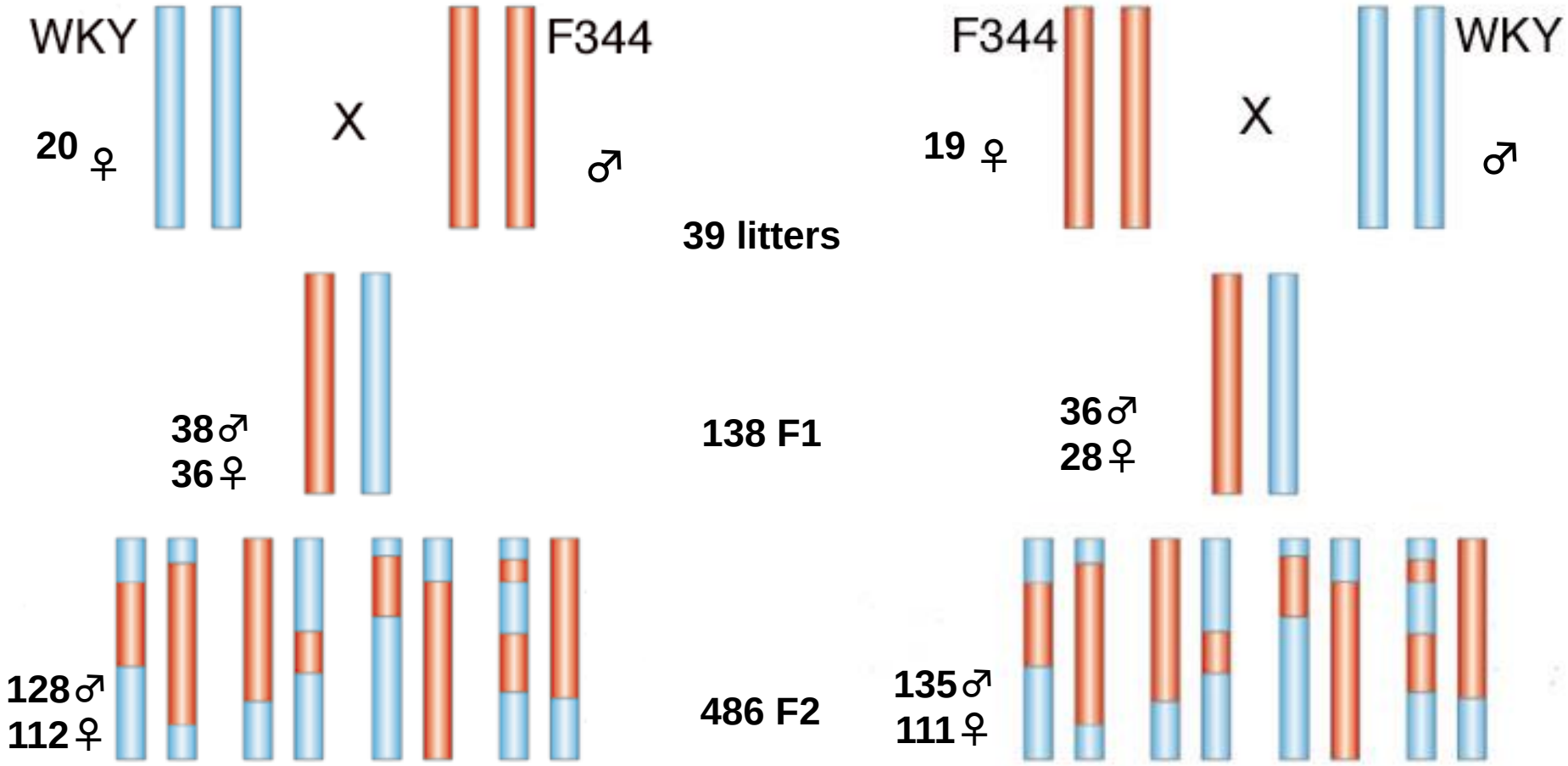
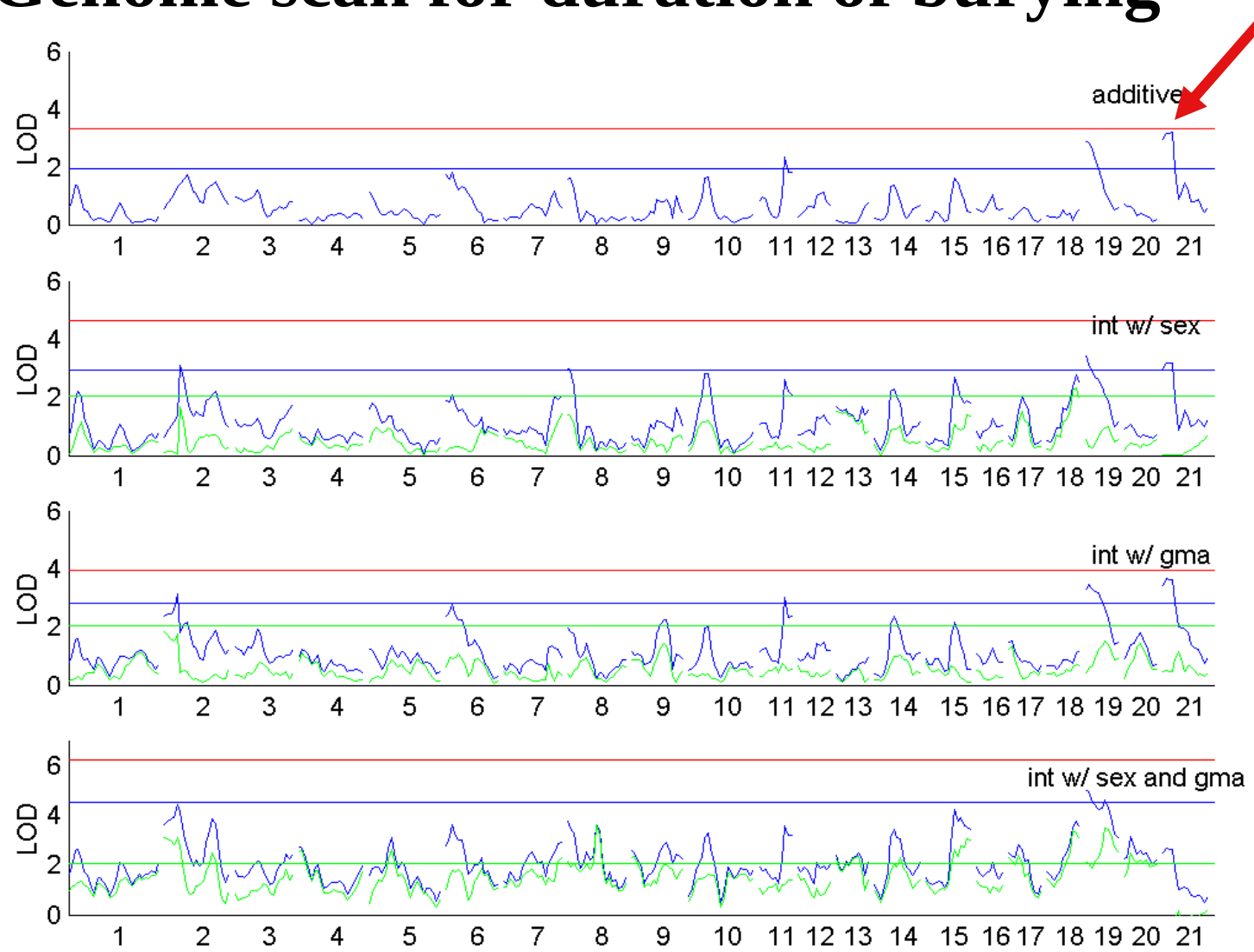
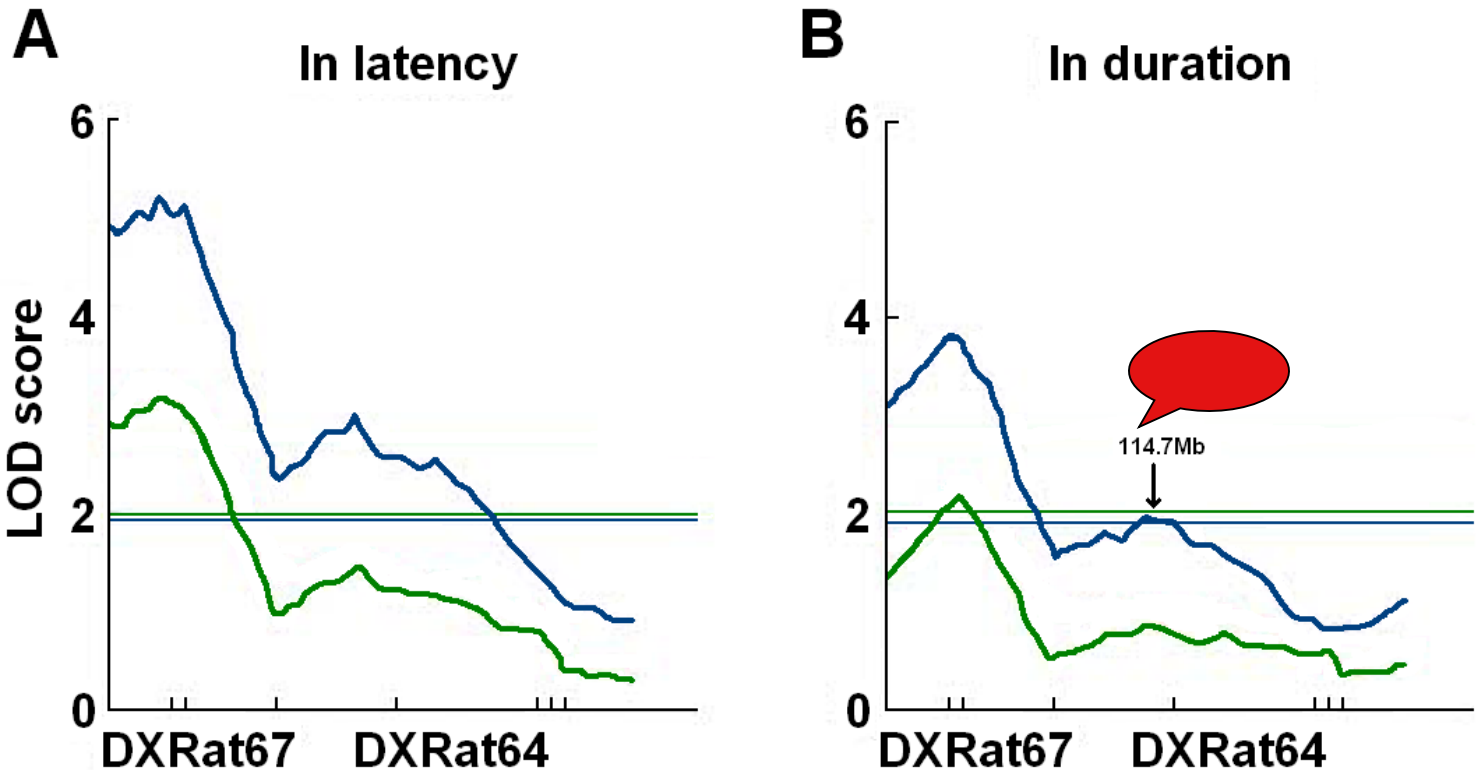
Quantitative Trait Locus Analysis



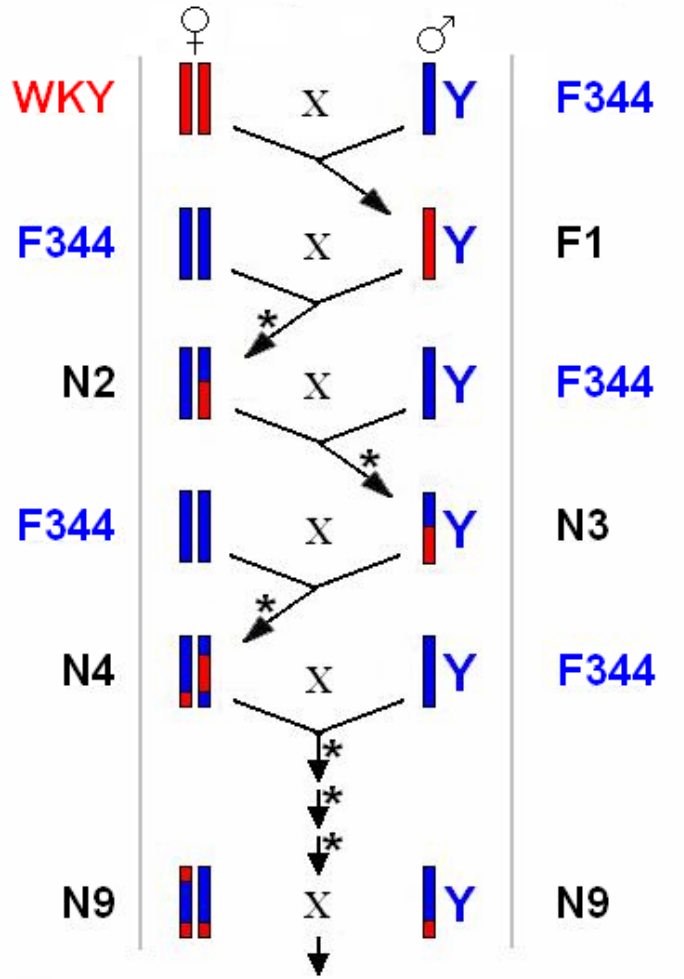
Genome scan for duration of burying





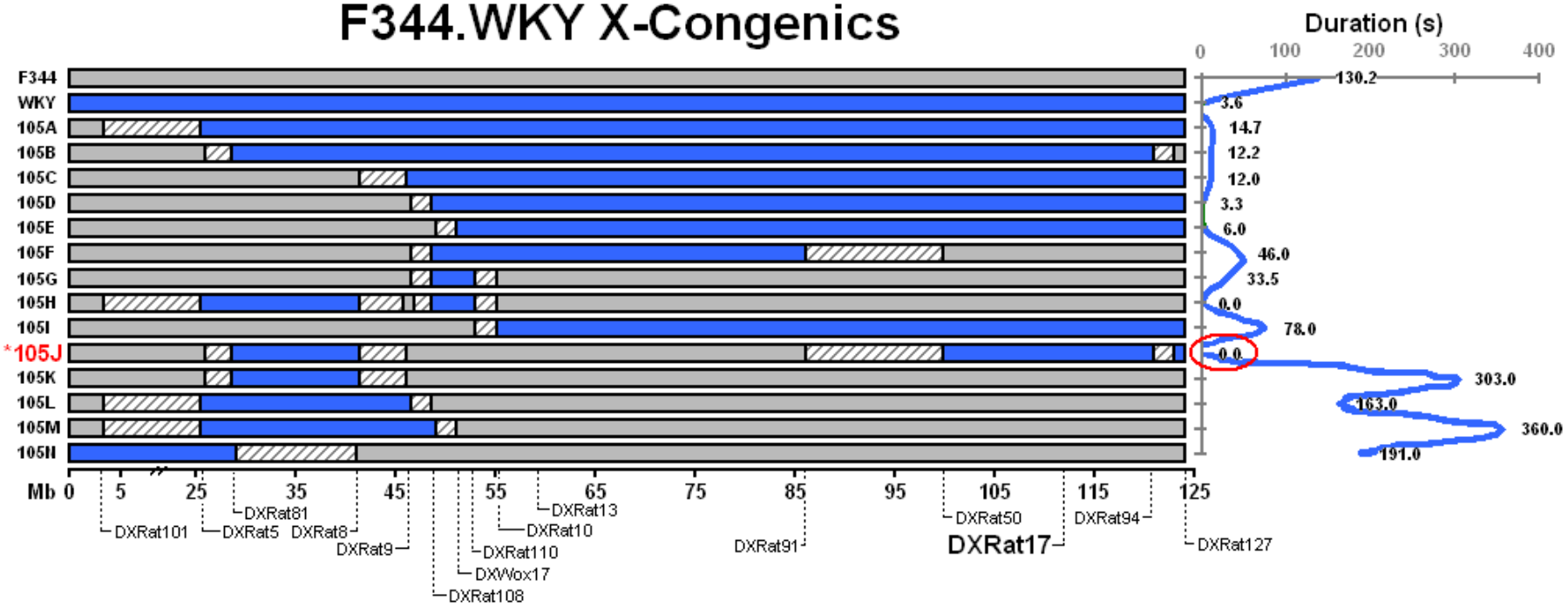
Blue: unadjusted, no covariates	Green: sex and lineage as additive covariates
(1) y = bo +b1q	(3) y = bo +b1x + b2q
(2) y = bo	(4) y = bo + b1x

X-Congenic Strain Construction

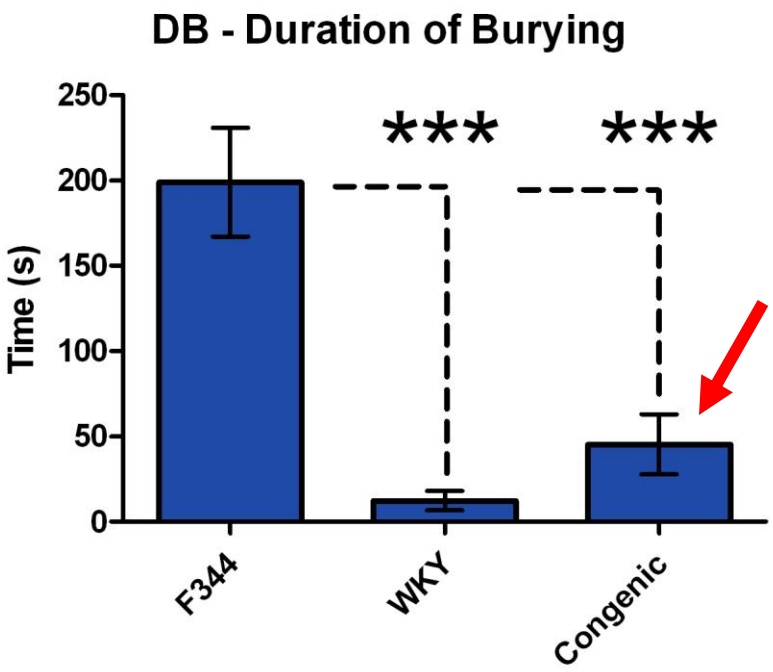


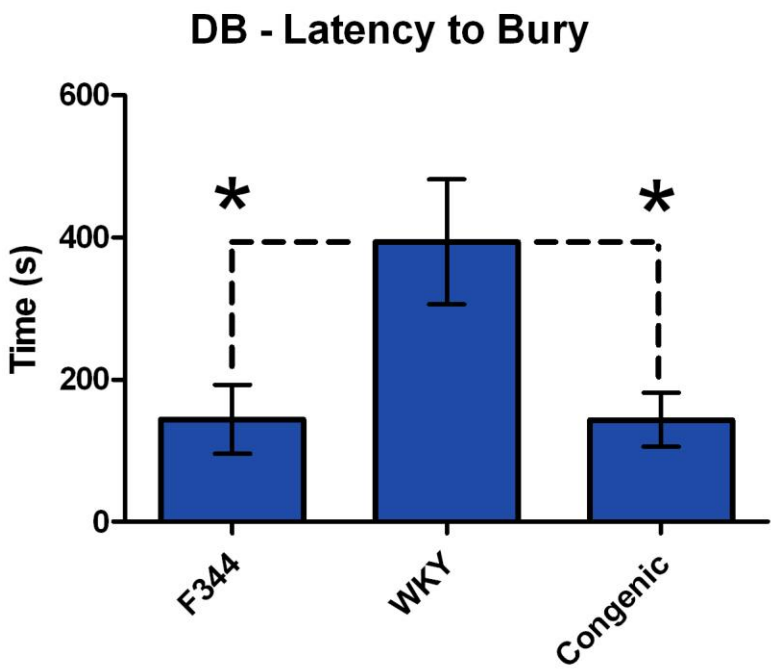
F344.WKY*(DXWox30-D1Got217)Eer*

* Selected for WKY *Stresp3*-locus and F344 genotypes on autosomes



*F344.WKY-(DXRat50-DXRat96)/Eer - preliminary congenic





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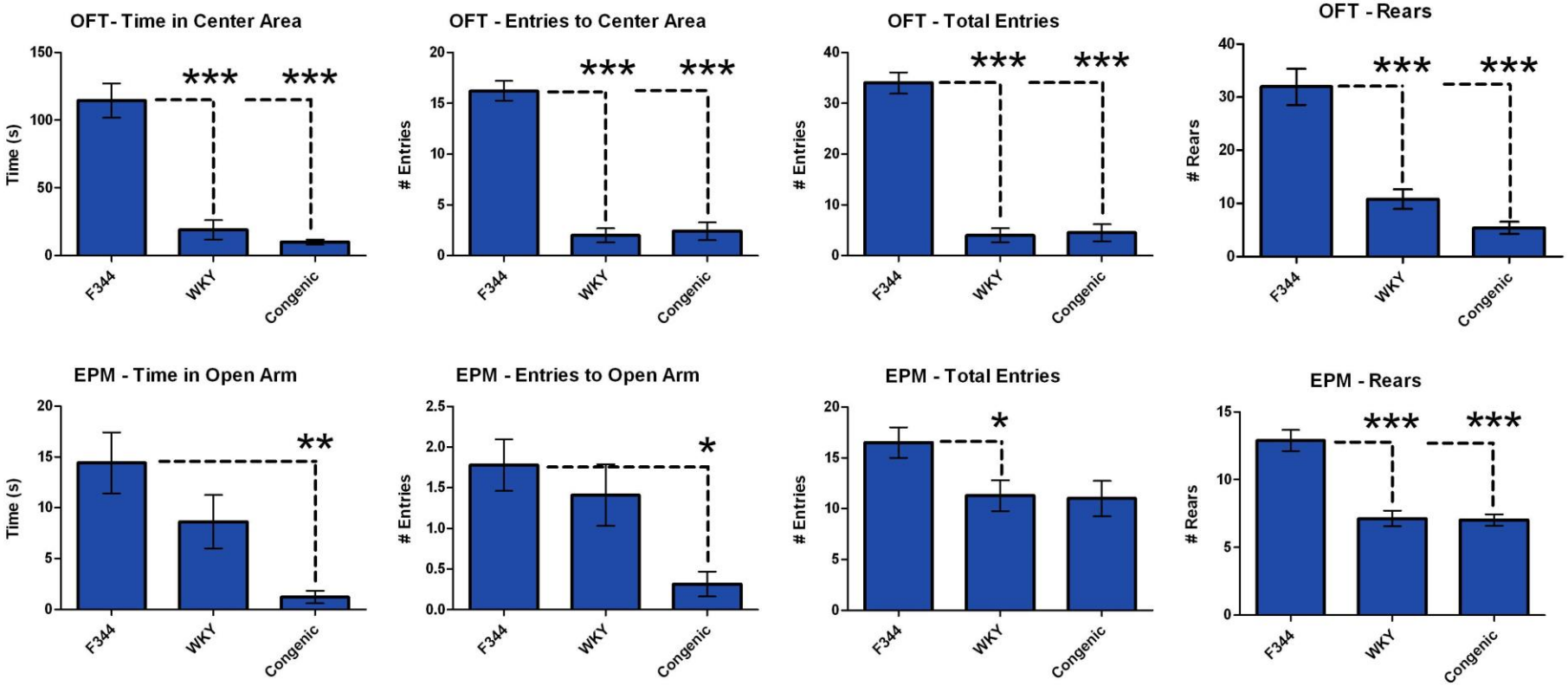

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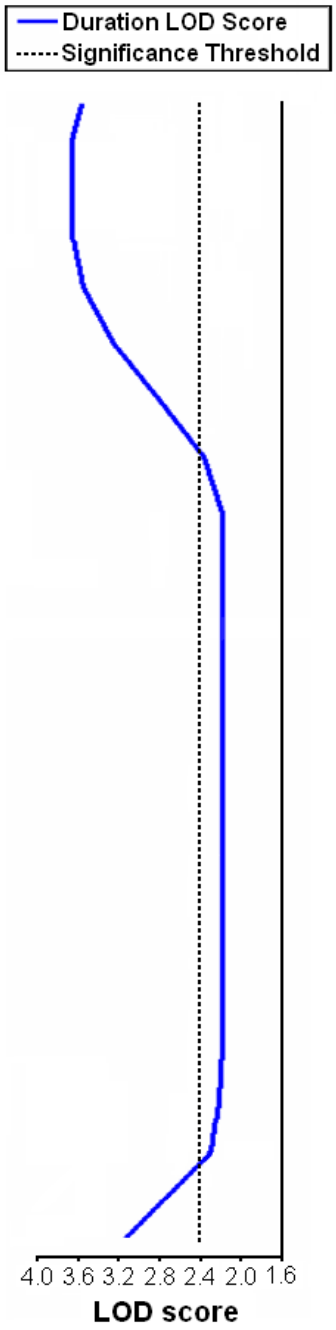

Genotype	Marker or SNP	Location (Mb)	Candidate Locations
F344		88,514,169	
?			← 1 - 4
?			
WKY	G/T	95,074,160	
WKY	A/G	95,487,735	
WKY	C/T	96,012,286	
WKY	C/T	96,457,403	
WKY	C/T	96,722,166	
WKY	A/T	98,285,593	
WKY	A/C	98,998,455	
WKY	DxRat50	99,773,357	
WKY	A/C	100,781,233	← 5
WKY	G/T	103,022,500	
WKY	C/T	104,799,115	← 6
WKY	A/T	106,125,462	← 7
WKY	A/G	106,474,065	
WKY	C/T	107,012,927	
WKY	C/T	107,227,193	
WKY	A/G	107,956,175	
WKY	C/T	108,613,039	
WKY	DxRat71	109,514,212	
WKY	A/G	110,819,544	
WKY	G/T	111,531,390	
WKY	A/G	112,109,283	
WKY	DxRat17	112,204,504	
WKY	C/T	112,483,874	
WKY	A/G	114,036,787	← 8
WKY	A/G	115,067,688	
WKY	A/G	115,467,035	
WKY	G/T	116,110,562	
WKY	A/T	116,944,293	
WKY	DxRat94	120,688,640	← 9 - 13
WKY	DxRat96	122,069,838	
?			← 14
F344		124,590,225	

Candidate	Brain Region	Fold Change
1.	Amygdala	-1.53
	Frontal Cortex	-1.42
	Hippocampus	-1.51
2.	Amygdala	-1.46
	Hippocampus	-1.56
3.	Amygdala	-1.20
	Hippocampus	-1.24
4.	Frontal Cortex	-1.19
	Hippocamous	-1.23
5.	Amygdala	-1.36
6.	Hippocampus	1.34
7.	Amygdala	-1.32
8.	Hippocampus	1.15
9.	Hippocampus	-1.22
10.	Hippocampus	-1.21
11.	Frontal Cortex	-1.16
12.	Frontal Cortex	1.11
13.	Frontal Cortex	1.12
14.	Amygdala	-1.34
	Hippocampus	-1.51

F344 Genotype
Marker/SNP = BN ≠ F344
Marker/SNP ≠ BN ≠ F344
Region with no Markers/SNP

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Conclusions:

- **QTL analysis of the reciprocal F2 intercross of WKY and F344 rat strains identified three loci on the X chromosome contributing to the variance in coping behaviors: *Stresp1*, *Stresp2* and *Stresp3*.**

- **A novel locus, at the proximity of *Stresp2*, emerged for duration of burying that is confirmed by generation of a congenic strain (X:88,514,169-124,590,225 Mb).**

- **Since hypoactivity is consistent with the decreased duration of burying phenotype, this congenic strain presents a consistent profile of inhibited or passive behavioral responses to environmental challenges.**

Conclusions (cont):

- **SNP analysis identifies regions/haplotypes that likely harbor genes responsible for these behaviors, as these regions differ from those of F344s and BN; both of which show active behaviors in these tests.**

- **Microarray results combined with the QTL analysis of the congenic strains and SNP-based haplotype analysis identified two major regions: at X:88.5-99.0Mb and at X:120.8-123.0Mb.**

- **Within these regions there are five potential QTGs that show significant expression differences between WKYs and F344s in more than one brain area.**

Outline

- Genetic contribution to individual coping strategies
  - Quantitative Trait Loci analysis
  - X-congenic strains
  - Genetic and transcriptomics: a combined analysis
- Genetic contribution to depressive behavior
  - Quantitative Trait Loci analysis
  - Genetic and transcriptomics: a combined analysis
  - Biomarkers for depression: a translational approach

Evaluation of Putative Endophenotypes for Major Depression

Table 1 Evaluation of Putative Endophenotypes for Major Depression[a]

	Specificity	State-independence	Heritability	Plausibility	Total
Psychopathological endophenotypes					
Depressed mood (mood bias)	+	+	0	+++	6
Anhedonia (impaired reward function)	+	+	+	+++	9
Impaired learning and memory	±	+	0	+	2
Direction of appetite change	–	±	+	++	4
Diurnal variation	++	+	+	++	6
Exec. cogn. function (response speed)	+	+	++	+	5
Psychomotor change	–	–	0	+	1
Increased stress sensitivity	–	+	++	+++	9
Biological endophenotypes					
REM sleep abnormalities	±	+	+	++	7
Increased amygdala activity	++	+	0	+++	6
Decreased subgenual PFC activity	+	+	0	+++	5
Left ACC volume reduction	+	++	0	++	7
Hippocampal volume reduction	–	++	+	++	6
Reduced 5-HT_{1A} receptor BP	+	++	+	+++	7
Tryptophan depletion	+++	+++	++	+++	14
Catecholamine depletion	+	+++	0	+++	7
dex/CRH test	±	++	++	+++	11
CRH dysfunction	+	+	+	+++	6

Hasler et al., 2004

Animal Models of Depression

Stress Models

- Stress induced
  - Chronic Mild Stress
  - Social Defeat
  - Prenatal Stress
  - Maternal Separation
- Tests for despair/depressive behavior
  - Forced Swim Test
  - Tail Suspension
  - Learned Helplessness

Genetic Models

- Selectively Bred
  - Maudsley reactive
  - Roman high and low avoidance
  - Flinders Sensitive Line
  - High swim/Low swim
  - **Wistar Kyoto More/Less Immobile**
- Transgenic models
  - Single candidate gene-background!!
- Inherent behavior
  - **Wistar Kyoto (WKY)**

Criteria for a Major Depressive Episode:

(at least 5 symptoms present in a 2-week period)	Modeled in animals	Modeled in WKY
•Depressed mood	No	
•Diminished interest or pleasure in activities	Yes	**Yes**
•Significant weight loss or gain	Yes	**Yes**
•Insomnia or hypersomnia	Yes	**Yes**
•Psychomotor agitation or retardation	Yes	**Yes**
•Fatigue or loss of energy/despair	Yes	**Yes**
•Diminished ability to concentrate	Yes	**?**
•Recurrent thoughts of death	No	

WKY as an animal model of depression

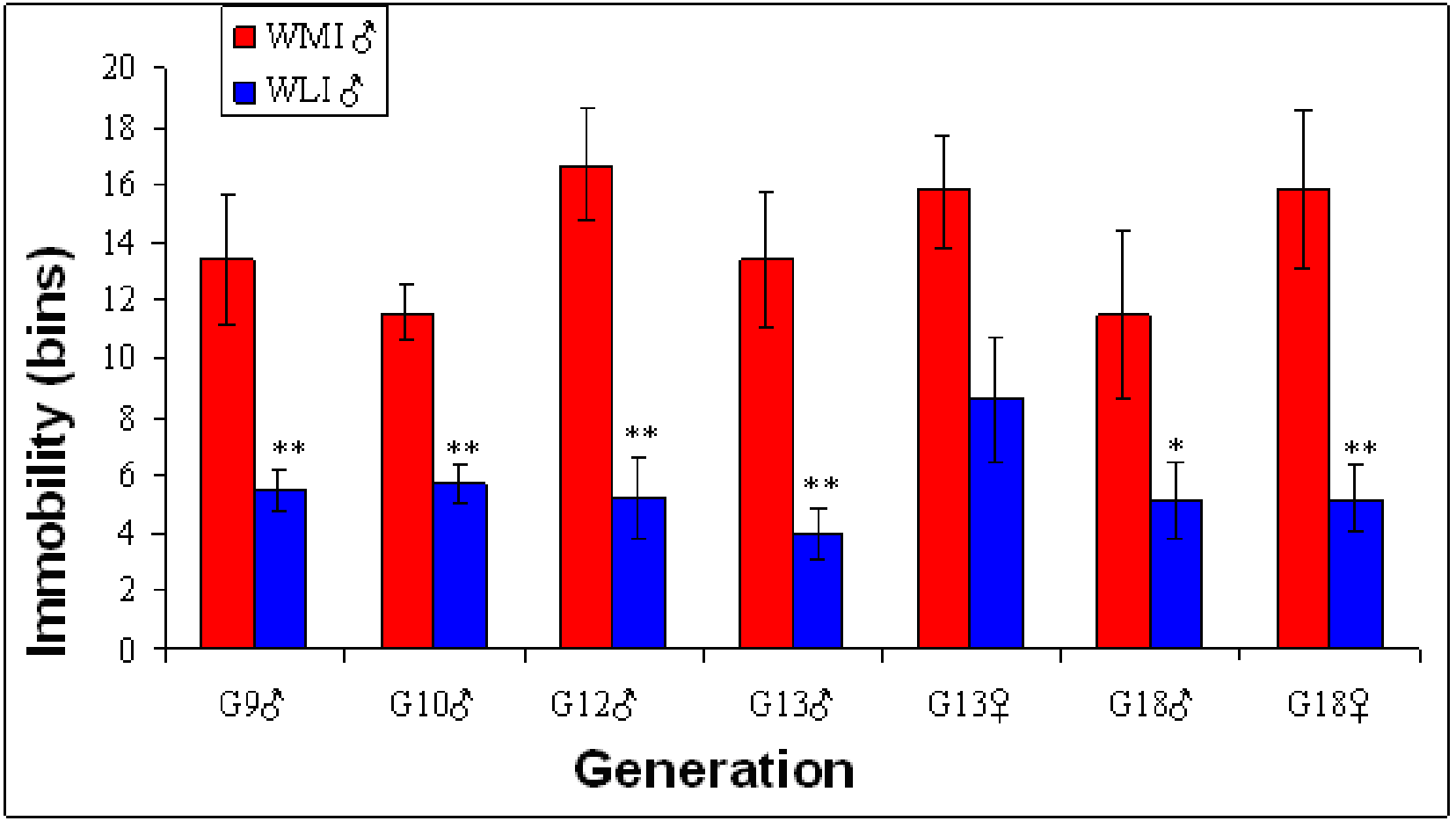
- **Exhibits behaviors analogous to those seen in depressed patients**
- **Depressive-like behavior is reversed after treatment with antidepressants (TCAs and MAOIs)**
- **Exhibits neuroendocrine and sleep changes similar to those seen in depressed patients**

Affimetrix microarray studies comparing gene expressions in:

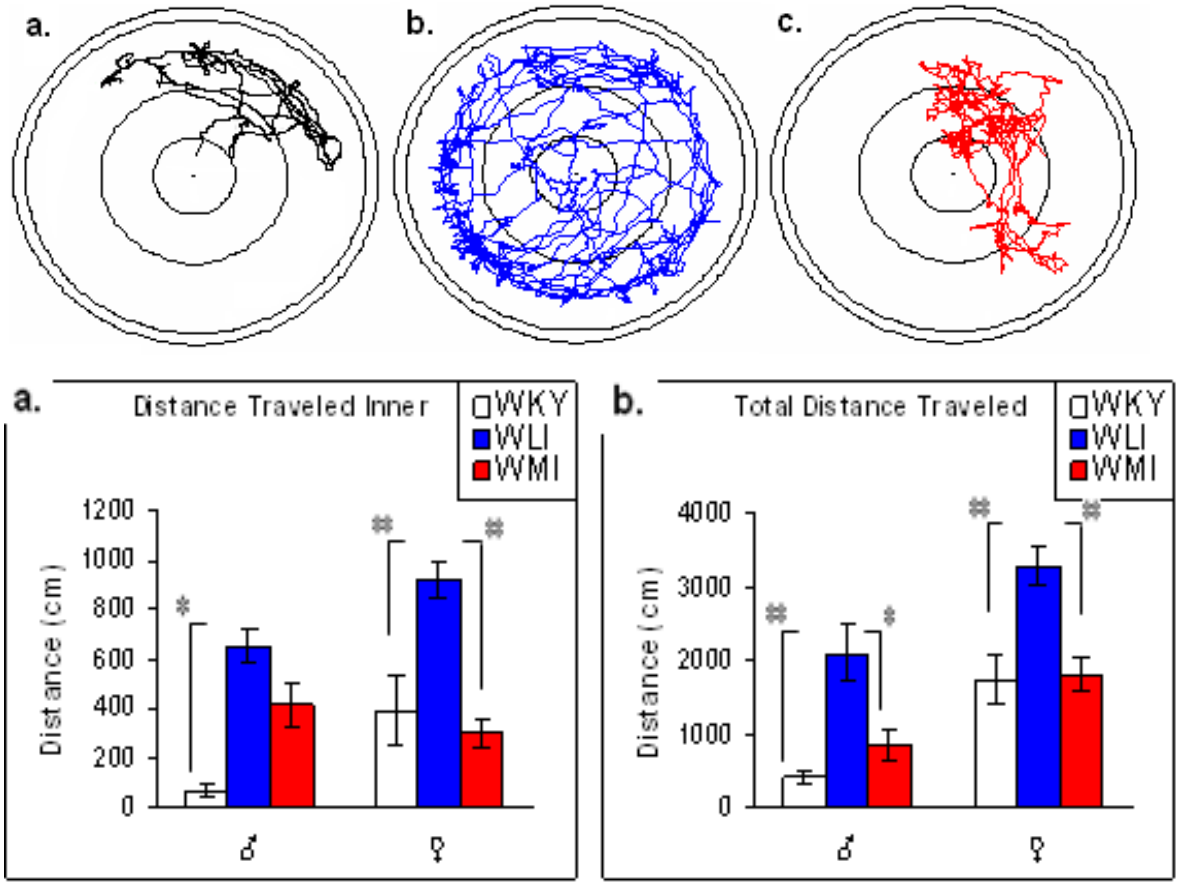
1. **amygdala, hippocampus, frontal cortex and striatum of male and female WKY and Fisher 344, WKY more immobile (WMI) and WKY less immobile (WLI) , chronically treated with vehicle or desipramine. (192 arrays)**

2. **in peripheral blood of the same animals**

Selectively bred Wistar Kyoto substrains



Behavior of WMI and WLI substrains in the open field test



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Strategy to identify biological markers for depression: a translational approach

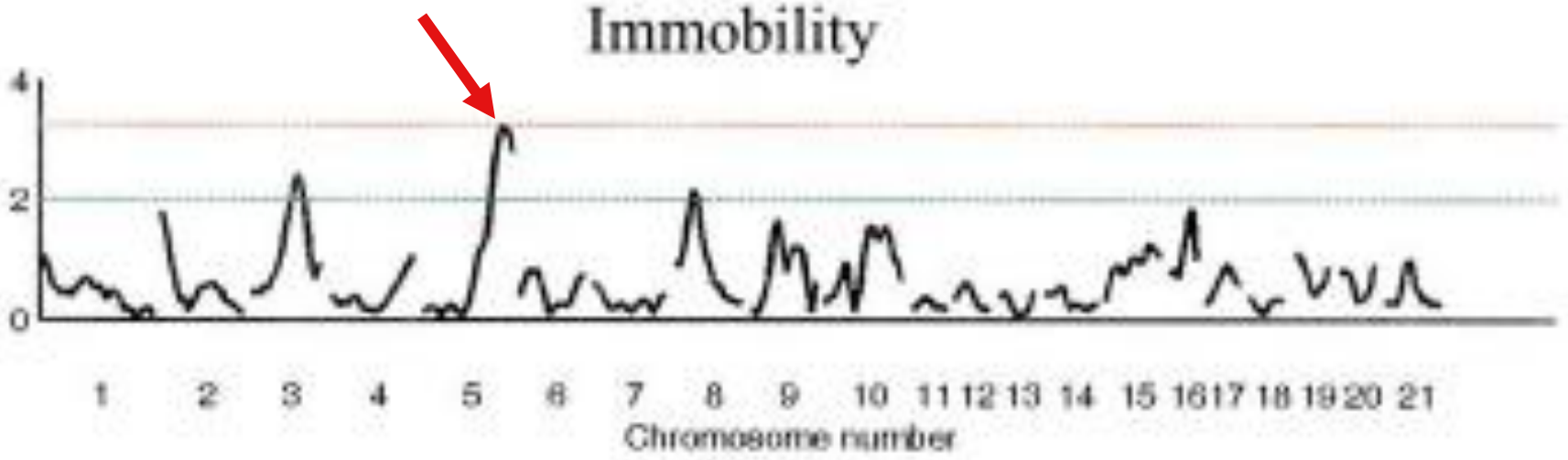
Genetic Markers

1. QTL of F2 WKY x F344 analyses identify locus
2. SNP analysis of parental strains compared to reference strain BN narrow regions
3. Selectively bred sub-strains
4. Microarray analyses of relevant brain regions between QTL strains and selectively-bred strains identify differentially expressed genes within these narrow regions
5. Differentially expressed genes mapped to synthenic region with human loci identified by GWA studies are candidates

Expression Markers

1. Selectively bred sub-strains
2. Microarray analyses of relevant brain regions AND BLOOD between selectively-bred strains identify genes differentially expressed in both BRAIN AND BLOOD
3. Validate candidate biomarkers in blood samples of depressed patients and controls

Despr3 locus synthenic to human depression and neuroticism locus at Chr 1:24,207,596 -42,694,514bp



Identification of potential QTGs
SNP analysis of *Despr3* locus

	Chr	bp	WKY	F344
G/T	5	1407384 59	0	2
C/T	5	1411734 87	2	0
A/G	5	1425019 22	2	0
C/G	5	1425592 51	0	2
C/T	5	1432281 63	0	2
A/C	5	1434718 54	0	2
C/T	5	1436335 65	0	2
C/T	5	1449941 77	2	0
A/G	5	1450319 80	0	2
A/G	5	1475696 68	0	2
C/T	5	1477185 86	2	0
A/G	5	1501987 28	0	2
C/T	5	1503522 36	2	0
C/T	5	1505112 34	2	0
A/T	5	1509849 43	0	2
C/T	5	1517123 42	0	2
A/G	5	1528404 41	0	2

0=same as BN **2=differs from F344 and BN**

Candidate genetic biomarkers at Depr3 locus between WMI and WLI

Gene Title	Gene Symbol	Rat Chr 5		Human Chr 1	
		Start (bp)	Stop (bp)	Start (bp)	Stop (bp)
Stromal membrane-associated protein 1-like	Smap1l	141544855	141590838	40612315	40661581
Mitochondrial ribosomal protein S15	Mrps15	145397725	145408655	36702556	36693960
Similar to RIKEN cDNA 1810007P19 / Organic solute transporter protein 1	RGD1306596/OSTI	145410856	145441425	36656095	36688695
Similar to 2610027C18Rik protein	RGD1308876/ Fam176b	145502833	145504274	36562342	36560295
Collagen alpha-2(VIII) chain precursor	Col8a2	145679611	145682255	36333433	36338433
serine incorporator 2	Serinc2	149279316	149301855	31658550	31680122
pumilio 1 (Drosophila)***	Pum1	149651257	149530800	31311151	31176900
Hypothetical protein LOC688757 / transmembrane protein 200B	LOC688757/ Tmem200b	150777938	150780338	29318526	29323008

*** all three brain regions, highlighted genes are primary candidates

Candidate genes at Despr3 locus between WKY and F344

Gene Title	Gene Symbol	Rat Chr 5		Human Chr 1	
		Start (bp)	Stop (bp)	Start (bp)	Stop (bp)
Similar to schlafen-like 1	LOC500540/ Slfnl1	141129	98141133769	41176358	41620940
similar to RIKEN cDNA 3110037I16/ chromosome 1 open reading frame 176	RGD1309802/ C1orf176	141507	6831415091 54	40747000	40754799
stromal membrane-associated protein 1-like	**Smap1l**	**141544**	**5141590968**	**40612315**	**40661581**
CAP, adenylate cyclase-associated protein 1 (yeast)	Cap1	142174	4411422011 20	40278842	40310908
hippocalcin-like 4	Hpcal4	142490	4621424983 59	39929676	39917232
RGD1559909/ chromoso me 1 open reading frame 122	RGD1559909/C1orf122	144180	8914418349	38046556	38047713
mitochondrial ribosomal protein S15	**Mrps15**	**145397**	**772145408365**	**36702556**	**36693960**
Collagen alpha-2(VIII) chain precursor	**Col8a2**	**145679**	**68114568252 5**	**36333433**	**36338437**
ADP-ribosylhydrola se like 2	Adprhl2	145685	4951456911 73	36327073	36332120
tyrosyl-tRNA synthetase	Yars	148350	3631483789 89	33005620	33013427
serine incorporator 2	**Serinc2**	**149279**	**13614930180 5**	**31658550**	**31680112**
syndecan 3	Sdc3	149487	9381495189 17	31154067	31118567
Hypothetical protein LOC688757/transmembrane protein 200B	**LOC688757/ Tmem200b**	**150777**	**39815078037 8**	**29318526**	**29323008**
YTH domain family 2	Ythdf2	151080	5991510893 93	28935723	28968874

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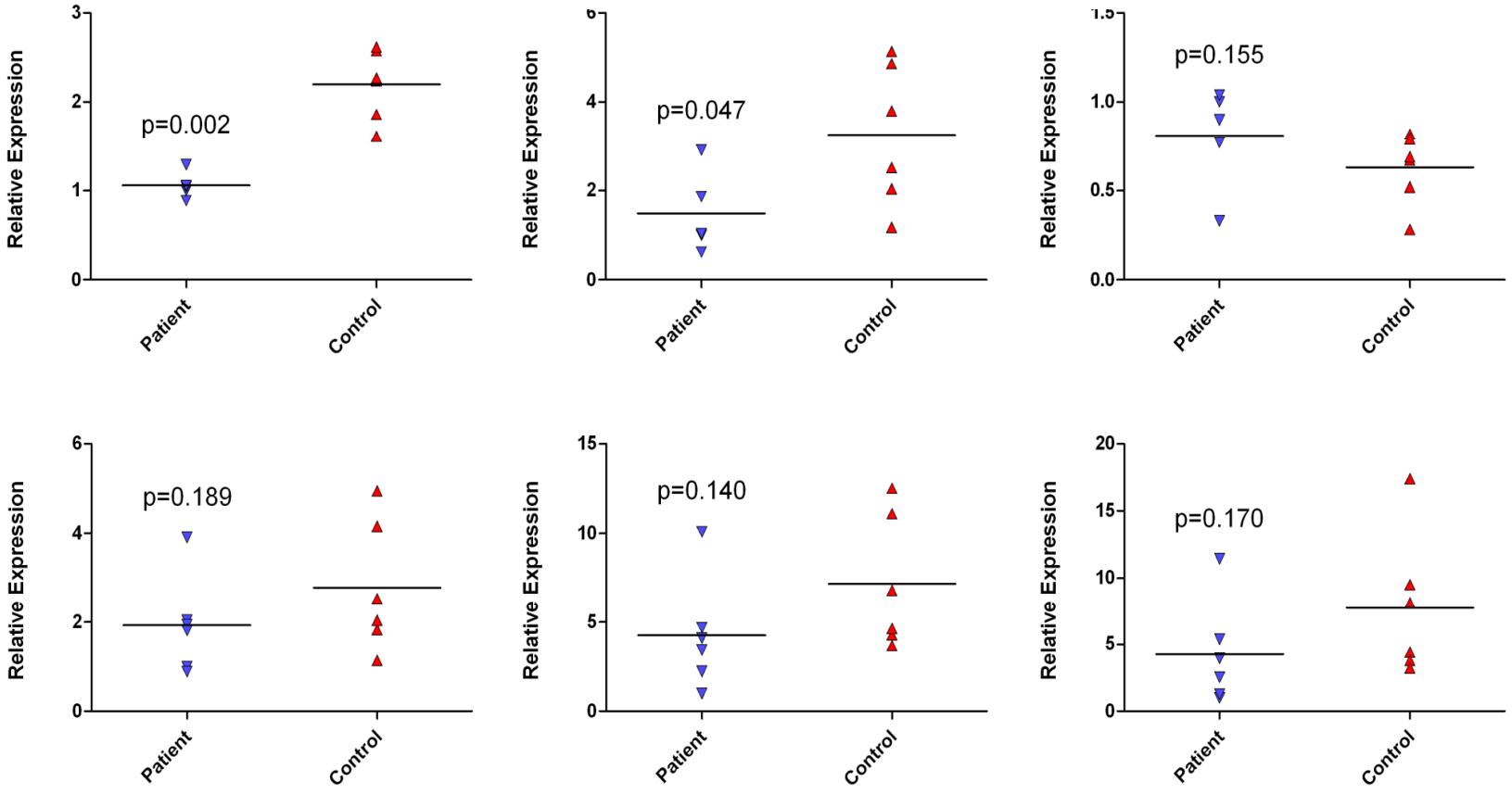
Candidate genes from expression profiling

- **155 genes whose expression was greater in WLI blood compared to WMI blood**
- **61 genes whose expression was greater in WMI blood compared to WLI blood.**
- **101 genes differed in both brain and blood between the two sub-strains.**
- **25 genes showed the greatest difference in both brain regions and blood: these genes are our primary candidates for biomarkers**

Biomarker candidates for human depression



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Summary

- Identified novel genetic animal models of passive coping and depression
- Passive coping may underlie many mood disorders
- The combination of genetic and transcriptomic analyses provide candidate genes that contribute to these endophenotypes
- Preliminary evidence indicate that blood biological markers can be identified based on the animal model data.

Collaborators

- **Northwestern**

(past and present lab members)

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