Identification of Candidate Genes that Control Differences in HPNS Seizure Susceptibility

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BACKGROUND

Inherited differences in susceptibility to the HPNS seizure elicited by fast compression (167 msw min^{-1}) in heliox are controlled by a quantitative trait locus (QTL) on chromosome 17 (*Chr 17*) of the mouse. Here we report an attempt to characterize the QTL. The relevance for humans derives from the fact that they share ~99% of their genes with mice. And human and mouse genes are syntenically arranged so that genes may be identified across the two species. QTL studies for atherosclerosis, asthma, hypertension, bone density and others in humans and mice have found that the corresponding QTLs are located in homologous regions. The predictive value of QTLs in humans by their identification in mice is clear. Moreover, in cases where the causal gene that underlies a QTL for a trait phenotype in mice is known, the same gene can be seen to underlie the corresponding QTL in humans.

METHODS

A physical map of the relevant ~12 Mb *Chr 17* region comprising seizure phenotype data from a *BXD* recombinant inbred (RI) strain panel was constructed at the GeneNetwork's website www.genenetwork.org. Because the seizure is at root a CNS phenomenon, congruence of gene expression was investigated for genes in a region of the HPNS QTL and genes in the brains of BXD mice in the same chomosomal region using the mRNA database Hippocampus Consortium M430v2 (Jun 06) PDNN.

RESULTS

The HPNS QTL contains a broad peak and a sharply defined one. Peak activity of brain genes shows close correspondence in hippocampus (as well as one or more other brain regions), to the peaks of the HPNS QTL in the following way: The broad HPNS peak (~10Mb) contains peak activities of vacuolar protein sorting 52 (*Vps52*), ring finger protein 1 (*Ring1*), flotillin 1 (*Flot1*), all linked to the major histocompatibility *H2* "loci" in region *A (H2-Aa, -Ab1), D (H2-DMb1), K (H2-K1, Ke6),* and *T (H2-T17, -T23, -T24)*; in the saddle between the HPNS broad peak and the sharp peak lies the significantly expressed methylmalonyl-coenzyme A mutase (*Mut*); and exactly matching the sharp peak is ectonucleotide pyrophosphatase/phosphodiesterase 5 (*Enpp5*). A single nucleotide polymorphism (SNP) analysis suggests that only *Vps52* and *H2-Aa* may be sufficiently polymorphic to be considered strong candidates.

Figure 1. Normal probability plot for *HPNS Fast*. Note the non-linearity (non-normal distribution) and clustering of BXD mean seizure threshold (atm) that are consistent with control of the variable by a QTL.

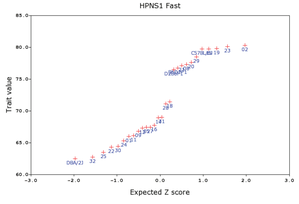


Figure 2. Genome-wide interval mapping for HPNS seizure threshold from WebQTL. Chromosome numbers are along the top of the Figure. The Log Ratio Statistic (LRS) values across the 19 autosomes and the X chromosome, indicated by the wavy blue line, summarize the strength of association between variation in the HPNS seizure phenotype and the two genotypes of all markers and the intervals between the markers. Chr 17 harbors at least one HPNS-associated QTL. The red line indicates C57BL/6 (B6) alleles increase HPNS seizure threshold while DBA/2 (D2) alleles (green line) decrease it.

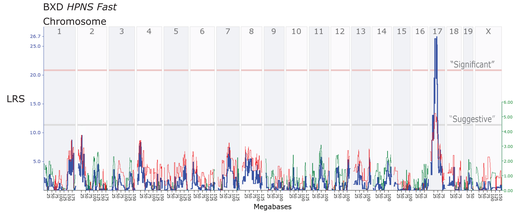
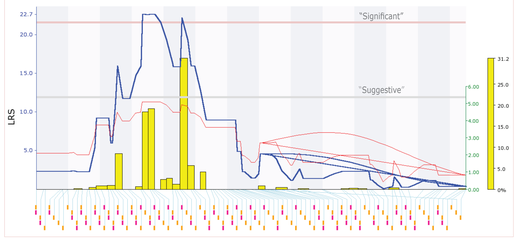


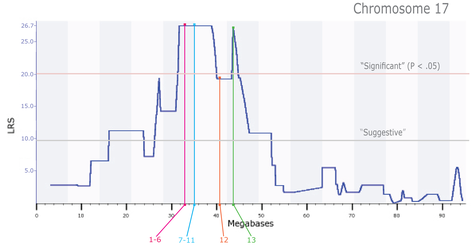
Figure 3. Chr 17 genetic map that is scaled centimorgans (recombinations per 100 gametes). As in **Figure 1**, the wavy red line indicates that increase in HPNS seizure threshold is associated with B6 alleles. The yellow histogram bars summarize the results of a whole-genome bootstrap of the HPNS trait that is performed 1000 times to provide additional positional precision. The colored blocks under the X-axis denote the relative positions of SNP loci that are highly significantly associated with the BXD *HPNS Fast* phenotype by WebQTL's GeneNetwork.



LRS (LOD)	SNP Locus	Gene	Probe Set	Chr 17 position (Mb)
26.384 (5.723)	rs13482963	Vps52	1447894_x_at	33.577317
	rs6395893	Ring1	1422647_at	33.631601
		H2-Ke6	1454987_a_at	33.636502
		-DMb1	1449580_s_at	33.759289
		-Ab1	1451721_a_at	33.87553
		-Aa	1452431_s_at	33.891537
		Flot1	1448559_at	35.438721
26.384	rs3672987	H2-K1	1425336_x_at	34.875152
	rs13482958	-T24	1422160_at	35.61401
	gnf17.035.152	-T23	1449556_at	35.71728
	mCV22965443			
	rs13482963			
26.384	rs6242153	H2-T17	1449875_s_at	35.646496
	rs13482948			
26.732 (5.799)	rs13482998	Enpp5	1425702_a_at	43.548812
	rs3677240			

Table 1. This table shows that peak LRS for SNPs that are highly significantly associated with the HPNS trait phenotype and with positional candidate genes represented by the **Hippocampus Consortium M430v2 (June 06) PDNN Database** probe sets for the BXD lines: Genes 1-6 (*Vps52, Ring1, H2-Ke6, -DMb1, Ab1, Aa*) as well as gene 7 (*Flot1*) with the same two SNPs (rs13482963 and rs6395893). These genes are also associated almost as strongly (near-peak LRS values) with the SNPs whose LRS values are maximal for genes 8 - 11 (*H2-K1, -T24,* and *-T23*) and vice versa. This is probably due to linkage in a strongly conserved region of Chr 17. The situations for gene 12 (*Mut*) and 13 (*Enpp5*) are that there are no polymorphic SNPs in the BXD set for *Mut*, and only 2 for gene 13 (*Enpp5*) although there are many present in BXD progenitor inbred strains other than C57BL/6 and DBA/2.

Figure 4. Chr 17 interval map showing peak expansion. This is a physical map in which the X-axis scale is based on DNA length measured in nucleotides or base-pairs. In the roughly 12 Mb region that encompasses the HPNS QTL(s) there are about 180 positional candidate genes, but their biological and bioinformatics plausibility vary greatly. The vertical colored lines show the approximate positions of 13 candidate genes that map to the HPNS QTL interval and also are significantly coexpressed. Information about the genes is presented in **Table 1**.



	Gene Symbol	Gene Name	Position Chr 17 (Mb)	Gene Length (Kb)	BXD SNP Density (SNP/Kb)	BXD SNP Count	BXD SNP Non-redundant	BXD SNP Informative
1	Vps52	vacuolar sorting protein 52	33.566266	11.399	2.775	31	30	8
2	H2-K1	K1, K region	33.606474	4.237	0.003	11	2	1
3	Ring1	ring finger protein 1	33.631538	3.557	3.374	12	5	0
4	H2-Ke6	K region expressed gene 6	33.636488	2.118	2.470	5	4	1
5	H2-DMb1	class II, locus Mb1	33.746125	14.580	3.018	44	44	0
6	H2-Ab1	class II antigen A, beta 1	33.871432	6.153	0.486	3	3	2
7	H2-Aa	class II antigen, alpha	33.891095	5.020	7.570	38	38	12
8	Flot1	flotillin 1	35.431400	9.434	1.378	13	8	0
9	H2-T24	T region, locus 24	35.614707	13.857	0.000	0	0	0
10	H2-T23	T region, locus 23	35.638029	2.723	0.733	2	2	1
11	H2-T17	T region, locus 17	35.646496	4.275	0.001	3	3	1
12	Mut	methylmalonyl-coenzyme A mutase	40.398191	24.606	0.691	17	0	0
13	Enpp5	ectonucleotide pyrophosphatase/ phosphodiesterase 5	43.541924	7.769	0.910	7	5	2
					Total	178	154	28

Table 2. Column 3 shows the presence of linkage between genes 1 - 6 and between genes 7 - 11. The last three columns list, respectively, the total number of SNPs in the BXD set, the number of unique SNPs, and the number of SNPs localized to exons. The very small number of the latter, with the possible exceptions *Vps52* and *H2-Aa*, suggest that genes 2 - 5 and 7 - 11 as well as genes 12 and 13 are essentially identical by descent (that is, there is no polymorphic variation) and they are therefore less likely candidates to express variability in HPNS seizure susceptibility.

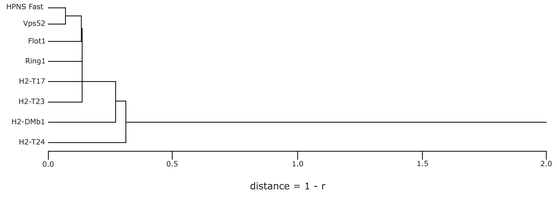


Figure 5. Hierarchical cluster tree of the traits *HPNS Fast, Vps52, Flot1, Ring1, H2-T17, -T23, -DMb1,* and *-T24*. Initially the plot is generated by computing distances between pairs of traits using (1-r) where r is the Pearson product-moment correlation. The hierarchy is assembled by successive linking traits and groups of traits.

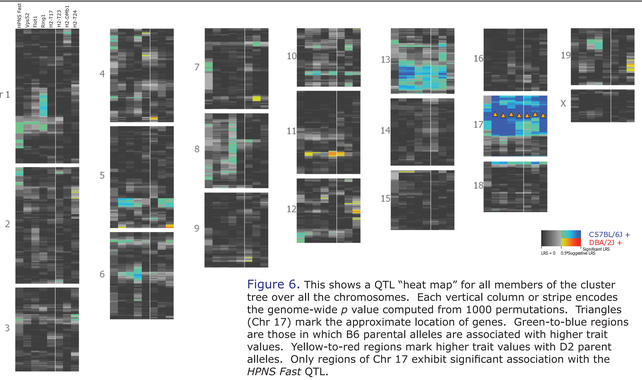


Figure 6. This shows a QTL "heat map" for all members of the cluster tree over all the chromosomes. Each vertical column or stripe encodes the genome-wide *p* value computed from 1000 permutations. Triangles (Chr 17) mark the approximate location of genes. Green-to-blue regions are those in which B6 parental alleles are associated with higher trait values. Yellow-to-red regions mark higher trait values with D2 parent alleles. Only regions of Chr 17 exhibit significant association with the *HPNS Fast* QTL.

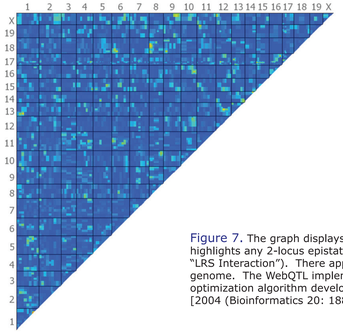


Figure 7. The graph displays pair-scan results for *HPNS Fast*. The plot highlights any 2-locus epistatic interactions (corresponding to the column "LRS Interaction"). There appear to be none of significance over the whole genome. The WebQTL implementation is based on the DIRECT global optimization algorithm developed by Ljungberg, Holmgren, and Carlborg [2004 (Bioinformatics 20: 1887-1895)].

CONCLUSIONS:

1 – Heritable difference in susceptibility to the HPNS seizure under fast compression in heliox among BXD mouse strains is associated with a QTL on *Chr 17*.

2 - The QTL is located in a ~12 Mb region (31.5 - 43.5 Mb) that contains about 180 positional candidate genes. Congruent expression of genes in the QTL region and genes in the hippocampus of BXD mice reduces the number of candidate genes to 13, human homologues of which localize to a region of human chromosome 6.

3 - The region containing the QTL appears on the basis of SNP analysis to be strongly conserved. Only 2 of the candidate genes (*Vps52* and *H2-Aa*) may be sufficiently polymorphic for parental B6 and D2 alleles to be associated with seizure threshold differences.

4 - *Vps52* and *H2-Aa* appear to be cisQTLs, meaning that each is closely linked to the gene whose transcript is the measured trait.

5 - *Vps52* may be a high priority candidate in respect of strength of correlation with the *HPNS Fast* trait and activity profile of genes in the hippocampus of BXD strains.

Caveat: These conclusions must be viewed as preliminary owing to the relatively small size of the sample of BXD strains for which HPNS seizure threshold data exist presently.