HPNS seizure risk: A role for the Golgi-associated retrograde protein complex?

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ABSTRACT

Previous attempts to characterize the genetic contribution to differential risk of developing the HPNS seizure in a mouse model system are extended to additional data and an analytical mode that incorporates the set of linked resources for systems genetics in the GeneNetwork project. A quantitative trait locus (QTL) affecting HPNS seizure phenotype was mapped to a ~6 megabase (Mb) gene-rich region of Chr 17 based on the degree of expression covariation among genes in the region of the QTL and genes in the brains of BXD recombinant inbred mice in the same chromosomal region. Use of GeneNetwork’s WebQTL analytical modules revealed that among >220 positional candidate genes, vacuolar protein sorting gene 52 (Vps52) has highest priority. It appears that a single nearly null mutation in a distal region of Vps52 3’UTR (untranslated region) defined by a DNA probe set is associated with >60% of the seizure risk difference between the high- and low-risk strains DBA/2 and C57BL/6, respectively. Based on the known contribution of the elements of the GARP complex – Vps52, -53 and -54 – to motoneuron abnormalities, mutation-depleted Vps52 may be implicated in HPNS seizure risk variation in the mouse and, by gene homology, also with human VPS52.

INTRODUCTION

All vertebrates tested, when exposed to increasing pressure in closely regulated helium/oxygen (heliox) atmospheres, experience heightened CNS excitability culminating at some threshold pressure in a convulsive seizure that is integral to high pressure nervous syndrome [1,2]. HPNS, although well characterized, presents a problematic feature common to attempts to understand the associated phenomena in humans – the presence of interindividual variation that is, at least in part, heritable [3,4].

Some degree of heritability is indicated in the stability of HPNS symptom development and progression in individuals exposed multiple times to the same compression conditions, and the presence of three groups differentiated electrophysiologically in the power of theta activities [4]. Establishing inheritance modes is difficult – especially for the seizure – in light of constraints on experimental manipulation of humans and the large samples required.

The earliest systematic study of the sources of variability in the development of HPNS among non-human species contains the explicit suggestion of a genetic (heritable) basis for control of HPNS seizure susceptibility [5] in the combination of a large range of mean convulsion thresholds with small intrastrain variance relative to interstrain variance among inbred strains of mice as representing “intrinsic biological variation” under a constant compression schedule. Inheritance of HPNS seizure susceptibility differences in inbred mice and derivative generations using a statistically robust modeling procedure produced evidence of a quantitative trait locus (QTL) of major effect (essentially a Mendelian element) localized to Chromosome (Chr) 17 [6]. A 1991 reanalysis of published data from [6] in [7] using a recombinant inbred (RI) QTL approach to detect identifiable multigenic influences among a larger set of genetic markers named, in addition to the major element on Chr 17, QTLs of minor influence on Chrs 1, 2 and 12.

Eighteen years later, to underscore the extent to which genetic analysis of HPNS phenomena has lagged, the present study reanalyzes the only report of “genetic dissection” of HPNS seizure variation [6]. However, this study is extended to include data, first described here,
from 10 additional RI strains of the BXD set produced by well-defined crossover events from crosses between C57BL/6J and DBA/2J parental strains. Use of new techniques and thousands more genetic markers in a completely sequenced murine genome bids fair to implicate genetic and, perhaps, physiological bases for the HPNS seizure phenotypes. Human-mouse comparisons may now provide the means to judge the usefulness of the mouse as a model of human responses to high hydrostatic pressure.

METHODS

Experimental conditions

Data were collected on HPNS seizure phenotype from 34 BXD RI mouse strains (10 mice per strain) compressed at 100 MPa hr\(^{-1}\) (~1000 atm hr\(^{-1}\)). The sample represents the original set [6] but extended by an additional 10 BXD strains (unpublished). Data from the original BXD set (10 mice per strain) compressed at 10 MPa hr\(^{-1}\) (~100 atm hr\(^{-1}\)) are included in the analysis. All details of IACUC-approved conditions of compression and animal husbandry conform to those described in [6].

Statistical analysis

Analysis of results relied upon the methods made available to researchers by the set of linked resources for systems genetics from The GeneNetwork project at the University of Tennessee Health Science Center (www.genenetwork.org). WebQTl is the project’s modules optimized for online analysis of gene-environment combinations [8,9,10].

Detection of a QTL, polygenic inheritance of HPNS seizure phenotype that varies in degree of effect and can be attributed to interactions between two or more genes and their environment, comes from the form of a normal probability plot. In such plots the Z score of an RI strain is based on its ranking in 34 BXD RI strains versus actual expression level in the pressure phenotypes.

QTL mapping is accomplished through WebQTl, calculating millions of linear regression equations and assembling the results over the 19 mouse autosomes and the X chromosome treated as one overall length. This provides estimates of the strength of association between the trait of interest and the two genotypes C57BL/6 and DBA/2 for all markers and the intervals between them. Results are presented as values of the chi square statistic called the likelihood ratio statistic LRS. [LRS/4.61= LOD (likelihood of the odds)]. In selecting traits of interest, consideration was given to both BXD RI expression levels in candidate gene regions defined by oligonucleotide probe sets representing varied tissues, and the degree of concordance with HPNS phenotype in the BXD set. On this basis, the hippocampus (dentate gyrus, CA1-CA3) is best. This is somewhat reassuring given the propensity for that part of the forebrain to be affected in seizures of several types [11].

Pair scan plots implemented in WebQTl that are based on the DIRECT global optimization algorithm [12] were used to detect the presence of 2-locus epistatic, i.e., context-dependent interactions.

Cluster trees were generated from plots of distances between pairs of traits computed using (1-r) where r is the Pearson product-moment correlation. Hierarchical clusters were fashioned from successive linking of traits and groups of traits.

RESULTS

The distribution of mean pressures that elicit the HPNS seizure in 34 BXD RI mouse strains subjected to fast compression in heliox (100 MPa hr\(^{-1}\)) reveals two distinct groups \((p<0.001)\). In the normal probability plot of the data (Figure 1, below), the non-linearity in the non-normal clustering of trait values is consistent with a significant degree of control by a QTL.

![Figure 1](Image)

**FIGURE 1** – Normal probability plot of mean HPNS seizure threshold in 34 BXD RI strains and their C57BL/6 and DBA/2 progenitors compressed in heliox at 100 MPa hr\(^{-1}\).

Genome-wide interval mapping results of the HPNS seizure phenotypic variable from WebQTl is presented in Figure 2 (facing page). A peak in LRS value (=78.1, equivalent to LOD=17.0) in the interval containing Chr 17 indicates the presence with high statistical significance of a QTL of major effect on the expression of differences in HPNS seizure in the BXD RI set.
A physical map of Chr 17 depicting the X-axis scale as a distance based on DNA length measured in nucleotides was created to show expansion of the LRS peak that contains the HPNS QTL sequence interval (Figure 3, below). The roughly 6 megabase (Mb) region comprising the peak is gene-dense – more than twice the average per Mb – with ~220 positional candidate genes, including many of the major histocompatibility (H-2) loci. However, the plausibility of many of the candidate genes is weakened by the absence of transcripts whose expression covaries with HPNS phenotype. The list of candidate genes is shortened to 11 on the basis of covarying expression in the Hippocampus Consortium M430v2 (June 06) PDNN Database probe sets for the BXD lines [12]: Vacuolar protein sorting gene 52 (Vps52), flotillin 1 (Flot1), ring finger

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**FIGURE 2**

**FIGURE 2 – Genome-wide interval mapping of HPNS seizure phenotype.** Chromosome numbers are along the top of the figure. The wavy blue line summarizes the strength of association between variation in HPNS seizure phenotype and the two genotypes C57BL/6 and DBA/2 of all markers and intervals between markers. The red line indicates C57BL/6 alleles increase seizure threshold while DBA/2 alleles (green line) decrease it.

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**FIGURE 3**

**FIGURE 3 – Chr 17 interval map showing expansion of the LRS peak of Figure 2.** All of the candidate genes map to the HPNS QTL interval between 33 and 39 Mb and are significantly coexpressed. The red line indicates that C57BL/6 alleles increase seizure threshold.
protein 1 (Ring1), and 8 from the H-2 major histocompatibility complex – H-2Ke6, -DMb1, Ab1, -Aa, -K1, -T17, -T23 and -T24. The region containing the QTL(s) appears from analysis of BXD single nucleotide polymorphisms (SNPs) to be strongly conserved. Only two of the candidates, Vps52 and H-2Aa, may be sufficiently polymorphic in informative, coding DNA (8 and 12 informative SNPs, respectively) for parental B6 and D2 alleles to be associated with HPNS seizure threshold differences. Vps52 and H-2Aa are cis-acting QTLs on the strength of evidence that each of the transcripts maps to its own gene’s location. That is, it is likely (but not certain) that control of the genes’ expression lies within the gene.

Of the two candidates Vps52 and H-2Aa, the former is of higher priority for major effect on inherited HPNS seizure susceptibility. When distances between pairs comprising HPNS Fast and each of the 11 “short-list” candidate genes considered singly were computed using 1-r, successive linking of traits and groups of traits assembled a hierarchical cluster tree placing Vps52 closest to HPNS Fast. In addition, the normal probability plot of probe set 1447894_x_at (Figure 4, below) reflects the effect on expression of a Mendelian element localized to the 3’UTR of the Vps52 sequence that is confirmed by test \( t=17.32; p<0.01 \). That is consistent with the report of the presence of a single QTL associated with ~64% of the variance in a C57BL/6-DBA/2 derived F2 comprising 120 animals tested for HPNS Fast susceptibility [6].

When mapped using the cluster map function of WebQTL, all of the eleven 25 base pair (bp) perfect-match oligonucleotide probes of the 1447894_x_at set show the cis-acting effect on the transcript. This suggests that cis-regulation probably does not result from a hybridization sequence effect. The C57BL/6-DBA/2 expression difference appears to be due to an almost null mutation in DBA/2. However, the well-overlapped probes in the Vps52 3’UTR nucleotide sequence over the 36 bp interval 34,103,808-34,103,843 (National Center for Biotechnology Information Genomic Build 37) contains three mutations in the DBA/2 compared to the C57BL/6 reference sequence at SNP Build 128 positions 34,103,811 (G→C), -813 (T→C) and -833 (C→T), none of which is represented in all of the probes. The effect of SNP overlap on hybridization is position-sensitive, being highest at the center of the probe and lower farther away. Mapping the expression of each of the 1447894_x_at probes produces (Figure 5, facing page) a high→low, left→right gradient of QTLs (LRS scores) for Probe_988195 (LRS=93.8) → Probe_472341 (LRS=17.2). It is therefore very likely that there is an unknown SNP or insertion/deletion (INDEL) in the sequence that affects the hybridization of all of the probes. The predicted location of the sequence change in DBA/2 is indicated in the box in the middle of Figure 5.

A genome-wide interval map was made of HPNS seizure phenotypes in a set of 24 BXD RI strains compressed at a slower rate (10 MPa hr\(^{-1}\)) under test conditions otherwise similar to those rapidly compressed (100 MPa hr\(^{-1}\)). The putative predominant effect of single-mutation-mediated depletion of Vps52 expression on HPNS seizure susceptibility at fast compression is not apparent at slower compression (Figure 6, facing page).

**DISCUSSION**

The study shows that there is a QTL of major effect on the HPNS seizure susceptibility difference between C57BL/6 and DBA/2 mouse inbred strains on Chr 17 in a region that contains the H-2 major histocompatibility complex. A prime candidate for the QTL is heritable variation in a distal 3’UTR DNA sequence of Vps52, though presently only predicted. The newly released BXD genotype file upgrade produced by a high-density Affymetrix array that has ~580,000 high-quality SNPs out of a total of 623,124 [13] does not show a new SNP in the position predicted. However, “smoothing” of the SNP file to retain only the most proximal and distal marker in the genotype file when three or more
markers have the same strain distribution pattern (SDP) reduces both the total number of SDPs and the rate of false discovery. This procedure can also eliminate some genuine single-nucleotide polymorphism SDPs [13]. For now, and until the transcript sequence in question is fully validated, retention of the status of \textit{Vps52} as a QTL of major effect on HPNS seizure variation seems warranted.

\textit{Vps52} is a component of the mouse GARP gene complex comprised of \textit{Vps52} (on Chr 17), \textit{Vps53} and \textit{Vps54} (both on Chr 11) [14]. The GARP complex in humans as well as mice is required for the trafficking of the acid hydrolase cathepsin D to lysosomes by enabling the recycling of the cation-independent mannose 6-phosphate receptor from endosomes to the trans-Golgi network [15]. Depletion of any part of the murine complex

\textbf{FIGURE 5}

\textbf{Oligonucleotide probe set 1447894}_x\textunderscore at overlap with the C57BL/6 reference DNA sequence for a \textit{Vps52}
distal 3'UTR region (also showing 3 DBA/2 variations). A potential SNP or INDEL position predicted for DBA/2 in thesequence is enclosed by the red box.

\textbf{FIGURE 6}

\textbf{Genome-wide interval mapping} for HPNS seizure threshold as described in Figure 2, but at a slower compression rate = 10 MPa hr\(^{-1}\).
leads to GARP mutant-specific missorting of a cathepsin D precursor to lysosomes that consequently swell, probably due to accumulation of undegraded proteins [16]. Defective vacuolar-vesicular protein sorting produces the motorneuron abnormality seen in the homozygous Wobbler mouse that results from a missense mutation in Vps54 that reduces its mRNA expression level [17,18]. It is suggested here that aberrant (low) mRNA expression in the DBA/2 Vps52 mutant 3’UTR control region may be associated with its lower HPNS seizure threshold (and therefore higher seizure susceptibility) relative to C57BL/6.

Many genes have assumed to have originated from single ancestral genes (orthologs [19]) in mice and humans code for protein products that are localized to vacuoles. When depleted or absent, they display neurological defects in mice but cause lethal disease states in humans at later life stages [20,21]. The suggestion is that the different phenotypes produced in the two species reflect a heightened sensitivity to vacuolar wastes and toxins in humans. This is related to a mass-specific metabolic rate that is ~12% that of mice and an average minimum time span to reach reproductive age that is ~150 times longer [22]. As a consequence, humans produce ~18 times the waste per gram of metabolically active mass until reproductive age.

The evolutionary response to a developing waste management problem posed by progressively increasing body size in the primate lineage leading to humans apparently took the form of accelerated sequence change [21]. Possible support for this idea in respect of evolution-augmented ability to deal effectively with cellular wastes in humans comes from consideration of the isoforms of Vps52. The mouse ortholog exists in three splice variant isoforms that produce “good” (functional) proteins in a 11.26 Kb sequence, whereas human VPS52 has 12 in its 21.77 Kb protein [23]. The presence in mouse Vps52 variants of two promoter sites and two polyadenylationA (polyA) sites suggests the possibility of alternative regulation of expression, but a relatively enhanced range of responses is possible in human VPS52 with its four promoters and five polyA sites.

Maintenance of sequence and (original?) functional similarity is present in reference sequences of Vps52 in both species that show: High sequence homology (>97%); formation of multi-subunit complexes with Vps53 and Vps54 that participate in the regulation of membrane trafficking events; and proteins of 723 amino acid length, 82.2 kDa mass, and pl 6.4 [24]. In this way, human similarity to mice in development of HPNS signs – perhaps including the seizure – may persist in some appreciable degree, while more complex and nuanced control of HPNS in humans arises from a larger array of heritable VPS52 phenotypes. This consideration is germane to analysis of the dynamism of HPNS seizure development evident in the apparent absence of a QTL of major effect at slower compression in mice (Figure 6). It suggests that “titration” of compression rates (high-to-lower) in animal experiments might disclose a gene action/interaction cascade of control elements identifiable by judicious use of sequential knockout mice originating from the Knockout Mouse Project [25]. Whether there are human orthologs that have the same or similar functions and interactions but nevertheless result in different arrays of mutant phenotypes is a question for future investigation.

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REFERENCES


